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(57) Abstract

A synthetic peptide capable of producing an immunological response to *C. trachomatis* in a vertebrate is disclosed. The synthetic peptide comprises at least one conserved T-helper cell stimulating epitope located within the peptide sequence ALNIWDRFDVFCTLGATTGYLKGNS and at least one B-cell neutralizing antibody stimulating epitope located within the peptide sequence FDVTTLNPTIAGAGDVK. In a preferred embodiment, the epitopes are colinear and in a particularly preferred embodiment the T-helper cell stimulating epitope is located closer to the N-terminus of the peptide than the B-cell neutralizing antibody stimulating epitope.

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SYNTHETIC PEPTIDE VACCINE FOR CHLAMYDIA TRACHOMATIS

FIELD OF THE INVENTION

This invention relates to the development and testing of vaccines for *Chlamydia trachomatis*. In particular, this invention is directed to synthetic peptide vaccines against *C. trachomatis* containing conserved B cell and T cell epitopes derived from the same protein.

BACKGROUND OF THE INVENTION

C. trachomatis is a causative agent of sexually transmitted diseases (STDs) which afflict an estimated 3 million people a year in the United States alone (Washington, et al., *JAMA* 257:2070, 1987). In women, *C. trachomatis* infection of the lower genital tract can ascend to the fallopian tubes causing salpingitis. Chlamydial salpingitis can lead to tubal blockage and cause infertility or ectopic pregnancy. It is estimated that in the United States 200,000 women per year become infertile as a result of chlamydial salpingitis. Measures to control or even prevent chlamydial STDs are badly needed.

Serotyping of *C. trachomatis* isolates separates them into 15 distinct serovars and three serogroups (Wang, et al., *Infect. Immun.* 7:356, (1973) and Wang, et al., *J. Infect. Dis.* 152:791, 1985): the B-serogroup consists of serovars B, Ba, D, E, L1, and L2; the intermediate serogroup, serovars, F, G, K, and L3; and the C-serogroup, serovars A, C, H, I, and J. Serovars D, E, F, G, H, I, J, and K are most commonly associated with chlamydial STDs. Greater than 80% of *C. trachomatis* caused STDs are due to infections caused by serovars D, E, F, or G (Kuo, et al., *Infect. Immun.* 41:865, 1983). The proportion of *C. trachomatis* serovars isolated in the Seattle metropolitan area between 1965 and 1982 was 46.5% (serovars D and E), 24.6% (serovars G and F) and 5-7% (serovars H, I, J and K) (4). This distribution of serovars has not changed over the past decade and is also representative of isolates obtained in other urban areas of the United States (Batteiger, et al., J. *Infect. Dis.* 159:661, 1989). Thus, a successful vaccine against chlamydial STDs must protect against multiple *C. trachomatis* serovars with coverage against serovars D, E, F, and G being vital.

The most promising antigen for the development of a vaccine against chlamydial STDs is the *C. trachomatis* circa 40 kDa major outer membrane protein (MOMP). It is the principle *C. trachomatis* serotyping antigen (Caldwell, H.D. and R.C. Judd., *Infect. Immun.* 38:960 (1982); Caldwell, H.D. et al., *Infect. Immun.* 31:1161 (1981); Caldwell, H.D. and J. Schachter, *Infect. Immun.* 35:1024, 1982) and is the only surface component to which chlamydial neutralizing antibodies have been described (Zhang et al., *J. Immunol.* 138:575 (1987) and Zhang et al., *Infect. Immun.* 57:636, 1989). The MOMP genes of several *C. trachomatis* serovars have been sequenced (Pickett, et al., *FEMS Microbiol. Lett.* 42:185

(1987), Zhang, Y.-X., et al., *Nucleic Acids Res.* 18:1061 (1990), and Hamilton, et al., *Nucleic Acids Res.* 17:8366, 1989) and are characterized by four symmetrically spaced hypervariable domains (VDs) that are flanked by regions of amino acid homology.

The MOMP VDs are the targets of species-specific chlamydial neutralizing monoclonal antibodies (mAbs) and Fab fragments prepared from neutralizing mAbs inhibit chlamydial infectivity by blocking their attachment to host cells (Su, H. and H.D. Caldwell. *Infect. Immun.* 59(8):2843, 1991). Furthermore, proteolysis of chlamydiae with trypsin leads to a loss in their ability to attach to host cells and is associated with cleavage within the surface exposed VDS of the MOMP. These findings strongly support a role for the MOMP VDs as surface structures which are important in the binding of chlamydiae to host cells. Thus, epitopes located within surface exposed MOMP VDs are rational targets for the development of a synthetic chlamydial vaccine.

Synthetic peptide vaccines incorporate T-helper (T_H) cell epitopes to enhance the immunogenicity of haptenic neutralizing B-cell epitopes and to evoke specific T-cell immunologic memory. By using overlapping synthetic peptides corresponding to the entire MOMP sequence in T-cell proliferation assays and as *in vivo* priming immunogens for the production of an anamnestic IgG antibody response, we showed that one peptide, termed A8, possessed functional T_H activity. We directly demonstrated that peptide A8 possessed functional T_H-cell activity by colinearly synthesizing it with the VDI sequence of serovar A, which contains a B-cell epitope, and showing that the production of IgG antibodies specific to B-cell epitopes within the VDl sequence was dependent on the A8 portion of the chimeric immunogen (Su, et al., *J. Exp. Med.* 172:203, 1990). Peptide A8 corresponds to MOMP amino acid residues 106-130. This region of the MOMP is largely sequence invariant among the different *C. trachomatis* MOMPs suggesting that the T_H-cell epitope contained within its sequence is antigenically conserved across serovars. While this synthetic peptide is useful for generating responses to serovar A, it does not generate protective responses to the other serovars.

Therefore, it is an object of this invention to prepare a synthetic peptide vaccine to a variety of C. trachomatis serovars that is suitable for generating protective immune responses in humans.

SUMMARY OF THE INVENTION

The present invention provides a synthetic peptide capable of producing an immunological response to *C. trachomatis* in a vertebrate comprising a conserved T-helper stimulating epitope from the major outer membrane protein of *C. trachomatis* and a serovar

conserved B-cell neutralizing antibody stimulating epitope from the major outer membrane protein of *C. trachomatis*. In a preferred embodiment of this invention, the T-helper cell stimulating epitope and the B-cell neutralizing antibody stimulating epitope are colinear. Preferably the peptide contains the sequence identified as SEQ ID NO:3. In one embodiment the synthetic peptide T-helper cell stimulating epitope is located within SEQ ID NO: 1 and in another embodiment the synthetic peptide is located within SEQ ID NO:2. In yet another preferred embodiment of this invention, the T-helper stimulating epitope is located within SEQ ID NO:1 and the B cell neutralizing antibody stimulating epitope is located within SEQ ID NO:2. Preferably the T-helper stimulating epitope located within SEQ ID NO:1 is on the peptide N-terminus side of the B cell neutralizing antibody stimulating epitope located within SEQ ID NO:2. In another preferred embodiment of this invention the peptide additionally comprises at least one species specific B-cell neutralizing antibody stimulating epitope. It is also contemplated that the synthetic peptide of this invention additionally comprises a known T-helper stimulating epitope from a protein other than the major outer membrane protein of *C. trachomatis*.

This invention also provides a method for inducing a protective immune response to *C. trachomatis* in a vertebrate comprising introducing a synthetic peptide comprising at least one conserved T-helper stimulating epitope located within SEQ ID NO:1 and at least one B-cell neutralizing antibody stimulating epitope located within SEQ ID NO:2 n a pharmaceutically acceptable buffer into a vertebrate and testing for neutralizing antibody to *C. trachomatis* in the vertebrate. In a preferred embodiment of this invention the introducing step comprises injecting the synthetic peptide intramuscularly.

Further, this invention additionally relates to a peptide for use in the preparation of a vaccine for *C. trachomatis*, comprising at least one conserved T-helper cell stimulating epitope located within SEQ ID NO:1 and at least one B-cell neutralizing antibody stimulating epitope located within SEQ ID NO:2, wherein said peptide is formulated in a pharmaceutically acceptable buffer for use as a vaccine.

Finally this invention also relates to an immunoassay for detecting the presence of antibody to *C. trachomatis* in a sample, comprising a synthetic peptide and means for detecting antibody bound to said peptide, wherein the amino acid sequence of said peptide comprises a conserved T-helper cell stimulating epitope located in SEQ ID NO:1 and a B-cell neutralizing antibody stimulating epitope located in SEQ ID NO:2.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graphic summary of studies assessing the immunogenicity of peptide A8-VDIV in B10 H-2 congenic mouse strains. Graph (A) details the serum IgG antibody response of mice immunized with peptide A8-VDIV. Graph (B) details the serum IgG antibody response of mice immunized with peptide VDIV alone. The mouse sera were tested against peptide A8-VDIV (dark block), serovar D MOMP (striped block), and peptide A8 (white block).

Figure 2 illustrates the results of the Pepscan ELISA analysis of the IgG antibody response of H-2 congenic mouse strains immunized with peptide A8-VDIV. All six mouse strains produced IgG antibodies reactive with octapeptides containing the septmeric species common LNPTIAG neutralizing B-cell epitope (Identified by the stippled pattern) contained within the A8-VDIV sequence.

Figure 3 is a comparison of the *C. trachomatis* serovar specificity of the antibody response of different H-2 congenic mouse strains immunized with peptide A8-VDIV. Sera from five mice were pooled, diluted 1:100, and tested by ELISA against formalin fixed elementary bodies (EBs). Results are expressed as optical density values at A_{405}.

Figure 4 is a comparison of the serum neutralizing activity of H-2 congenic mouse strains immunized with peptide A8-VDIV for three representative *C. trachomatis* serovars. Serum dilutions were incubated with chlamydia and inoculated onto monolayers of HaK cells to assay for chlamydial infectivity. The results are expressed as percent reduction in chlamydial infectivity.

Figure 5 illustrates the results of a Pepscan ELISA analysis of the IgG antibody response of monkeys immunized with peptide A8-VDIV.

Figure 6 is a comparison of the serum neutralizing activity of three cynomolgus monkeys immunized with peptide A8-VDIV for three representative *C. trachomatis* serovars following the procedures described for Figure 4.

Figure 7 is a comparison of the immunogenicity of synthetic peptides A8-VI and A8-VDIV in primates. Solid bars represent absorbance values (A_{405}) of pre-immune sera. The stippled bars are absorbance values of sera after immunization with the synthetic peptides.

DETAILED DESCRIPTION OF THE INVENTION

As used herein the term "synthetic peptide" is used to describe a linear sequence of amino acids produced by laboratory chemical synthesis schemes that is preferably less than

100 amino acids in length however it is additionally contemplated that these peptides could similarly be created by recombinant DNA technology.

We have previously described a neutralizing mAB, designated DIII-A3, that reacts by western blotting with the MOMPs of all *C. trachomatis* serovars except serovar K. Epitope mapping studies localized the DIII-A3 epitope to VDIV (Baehr et al., *Proc. Natl. Acad. Sci.* USA 85:4000, 1988) and fine mapping of the mAb identified the epitope as the septapeptide sequence ^{298}LNPTIAG^{304} within VDIV (Morrison, et al., 1992. "Immunology of *Chlamydia trachomatis* infections: Immunoprotective and immunopathogenetic responses." In: Sexually Transmitted Diseases. T.C. Quinn, ed. Raven Press, Ltd., New York, p. 57). The LNPTIAG sequence is invariant among *C. trachomatis* serovars except for serovar K where threonine replaces alanine at position 303. Although mAb DIII-A3 is broadly cross reactive with denatured MOMP by western blots, its immunoreactivity with intact *C. trachomatis* elementary bodies (EBs) by dot-immunoblot and its *in vitro* neutralizing activity is restricted to serovars within the B and intermediate serogroups indicating that this highly conserved epitope does not exhibit uniform surface accessibility among all *C. trachomatis* serovars. While the LNPTIAG neutralizing site is accessible on the surfaces of B and intermediate complex serovars, it is not seemingly accessible on the surface of all serovars and would thus, not be expected to be a suitable epitope for a widely protective *C. trachomatis* synthetic peptide vaccine.

As disclosed below, we found the chimeric peptide of this invention to be a good immunogen in both mice and primates, in that it: (i) targeted the production of neutralizing antibodies against the LNPTIAG B-cell epitope, (ii) was immunogenic in many mice strains disparate at H-2, and (iii) was a very effective priming immunogen for the production of an augmented IgG neutralizing response following secondary challenge with whole *C. trachomatis* organisms.

CONSTRUCTION OF THE SYNTHETIC PEPTIDE VACCINE

In a preferred embodiment of this invention, we have linked together the A8 sequence with the MOMP VDIV sequence which contains the antigenically common LNPTIAG neutralizing epitope, designated A8-VDIV, and have studied its immunogenic properties in both mice and primates. Methods for preparing the synthetic peptide are provided in Example 1. Peptide A8-VDIV corresponds to MOMP amino acids 106-130 (A8) and 293-309 (VDIV). Residues 106-130 (ALNIWDRFDVFCTLGATTGYLKGNS) contain a functional T_h-cell epitope. Peptide VDIV corresponds to MOMP residues 293-309 (FDVTTLNPTIAGAGDVK) and contains the sequence invariant LNPTIAG septmeric B-

cell epitope recognized by the *C. trachomatis* sub-species specific neutralizing monoclonal antibody DIII-A3. The chimeric peptide A8-VDIV was co-linearly synthesized with the A8 (T-cell site) sequence at its N-terminus and the VDIV sequence (B-cell site) at its carboxyl terminus. The A8-VDIV peptide was found to be a good immunogen in both species and preferentially targeted the production of antibodies to the LNPTIAG B-cell epitope. Importantly, the anti-peptide antibodies neutralized the *in vitro* infectivity of those *C. trachomatis* serovars that are epidemiologically important as causative agents of chlamydial STDs, suggesting that the oligopeptide may have considerable vaccine potential.

While the results described herein are associated with the B cell and T cell combination provided in Example 1, it is contemplated that the T-helper epitope and the B cell epitope could be combined in either order with the T-helper epitope placed either before or after the B-cell neutralizing epitope. Thus, in one preferred embodiment of this invention, the synthetic vaccine contains, begins at the amino end of the peptide with the T-helper epitope linked to the B-cell neutralizing epitope and in another preferred embodiment of this invention, the synthetic vaccine contains, beginning at the amino end of the peptide, the B-cell neutralizing epitope followed by the T-helper epitope. In addition, it is also contemplated within the scope of this invention, that the linking region spanning between the T-helper epitope and the B cell neutralizing epitope can incorporate any number of modifications known in the art. Thus, the peptide sequences incorporated as linking sequences between the epitopes of the vaccine could generate a flexible linking region, a rigid region, a hydrophobic region. a hydrophilic region or the like. Such modifications in the linking region between the conserved T-helper cell epitope and the conserved B-cell neutralizing epitope could be used to generate modified peptides suitable for testing as improved peptide vaccines for *C. trachomatis* using the testing strategy outlined below.

Further, other T-helper epitopes and B cell neutralizing epitopes could be combined to further advance the efficacy of the synthetic peptide vaccine. For example, it is contemplated that serovar specific neutralizing epitopes could be incorporated into the vaccine. The addition of a second *C. trachomatis* neutralizing epitope to this particular T-helper cell, B-cell neutralizing epitope combination could serve a number of functions. As one example, the additional neutralizing epitope could be incorporated into the vaccine to boost the efficacy of the vaccine for a particular serovar.

It is also contemplated within the scope of this invention that other T-helper epitopes could similarly be incorporated into the preferred combination of the T-helper and

B-cell neutralizing epitope of this invention. Other synthetic vaccines for other adventitious agents have incorporated T-helper epitopes from proteins from unrelated agents. For example, U.S. Patent No. 4,882,145 to Thornton, et al. disclose the incorporation of T cell stimulating regions of the Hepatitis B virus nucleocapsid protein as a method for enhancing the immunogenicity of a polypeptide immunogen.

It is further contemplated that the preferred synthetic vaccine of this invention, peptide A8-VDIV, can be incorporated in biodegradable microspheres such as those described by Eldridge, et al., (*Mol. Immunol.* 28:287, 1991 and *Infect. Immun.* 59:2978, 1991). Alternatively, it is additionally contemplated within the scope of this invention that the protective immunity of the A8-VDIV vaccine could be bolstered by the construction of recombinant cholera toxin B subunit A8-VDIV gene fusions using the methods of Sanchez et al., Schodel, et al., or Lipscombe et al. (*Proc. Natl. Acad. Sci. USA* 86:481, 1989; *Gene* 99:255, 1991; and *Mol. Microbiol.* 5:1385, 1991, respectively).

TESTING IMMUNOLOGIC ACTIVITY OF THE A8-VDIV CONTAINING PEPTIDE IN VIVO

Once the preparation of the synthetic peptide is generated, it is introduced into test animals such as mice, primates, or the like, to study the efficacy of the protective immune response of the vaccine. Vaccination schemes for mice and primates are provided in Example 2 and Example 3. Preferably the peptide is capable of inducing mucosal immunity, therefore it is contemplated that the peptide may be introduced through any oral or parenteral route known in the art. It is anticipated that following vaccine trials in experimental animals such as mice and primates, the vaccine will be introduced into humans to begin efficacy trials for the protection of *C. trachomatis* associated STDs. Those with skill in the art of vaccine development will be readily able to adapt the primate vaccine strategy into a strategy suitable for human vaccination.

There are a variety of methods known in the art for studying the B-cell neutralizing activity and T-helper cell induced antibody responses using in vitro tests. As one example of a method to study the helper function of the A8 portion of peptide A8-VDIV and to ascertain the MHC class II restriction in the recognition of the T_H-cell epitope(s) contained within the A8 sequence, we evaluated peptide A8-VDIV for its ability to elicit an IgG antibody response to the B-cell VDIV portion of the peptide in B10H-2 congenic mouse strains. Eight B10 congenic strains of mice disparate at H-2 were immunized with peptide A-8 VDIV or peptide VDIV alone. Following the secondary immunization, the mouse sera were tested by ELISA (as provided in Example 4) for IgG antibodies reactive to the free

peptides A8, VDIV, and whole MOMP (Fig. 1). Six of the eight mouse strains, (C57BL/10 ($H\text{-}2^b$), B10.A ($H\text{-}2^a$), B10.M ($H\text{-}2^f$), B10.WB ($H\text{-}2^{ja}$), B10.BR ($H\text{-}2^k$). and B10.SM ($H\text{-}2^u$), immunized with peptide A8-VDIV produced high titer IgG antibodies reactive with the VDIV peptide and whole MOMP (Fig. 1A). None of the mice produced IgG antibodies against the peptide A8 which contains the T_H-cell site of the oligopeptide immunogen. Two strains, B10.D2 ($H\text{-}2^d$) and B10.PL ($H\text{-}2^n$), failed to produce IgG antibodies reactive with peptide VDIV or MOMP following immunization with peptide A8-VDIV indicating that mice having these H-2 haplotypes were incapable of recognizing the T_H-cell epitope contained in the A8 portion of oligopeptide. Two of the eight strains of mice (C57BL/10 and B10.M) produced IgG antibodies reactive with peptide VDIV and MOMP following immunization with peptide VDIV alone (Fig. 1B), demonstrating that in addition to B cell epitopes the VDIV sequence also contains functional T_H cell epitope(s) whose recognition is restricted to the $H\text{-}2^b$ and $H\text{-}2^f$ haplotypes. Results are summarized in Table 1 below. Collectively, these findings showed that the T_H-cell epitopes contained within the A8-VDIV peptide are recognized by multiple MHC class II haplotypes and that these T-cell determinants provide cognate help for antibody production to B-cell epitopes located in the VDIV sequence.

TABLE 1

Immunogenicity of Peptide A8-VDIV in B10 Congenic Mice Differing at H-2

Mouse Strain	H-2 haplotype	ELISA IgG antibody titer: Peptide VDIV	ELISA IgG antibody titer: C. trachomatis serovar D	E	F	G	H	Peptide pin Mapping: response to (LNPTIAG)	Neutralizing antibody titer (ND_{50}): C. trachomatis serovar D	G	H
C57BL/10	(H-2^b)	1024	8192	4096	512	1024	64	+	256	128	<16
B10.A	(H-2^a)	256	4096	2048	64	128	<16	+	256	64	16
B10.D2	(H-2^d)	<16	16	16	<16	<16	16	-	<16	<16	<16
B10.M	(H-2^f)	256	4096	4096	512	1024	32	+	256	128	<16
B10.WB	(H-2^{ja})	1024	4096	4096	64	256	<16	+	128	32	<16
B10.BR	(H-2^k)	1024	8192	8192	1024	2048	16	+	256	128	<16
B10.PL	(H-2^u)	<16	64	16	<16	<16	<16	-	<16	<16	<16
B10.SM	(H-2^v)	128	1024	1024	64	256	<16	+	64	32	<16

The sera of the six responding strains of mice were tested by pepscan ELISA (see Example 5) to determine if the anti-VDIV antibodies produced were reactive with the targeted LNPTIAG B-cell epitope contained in the VDIV sequence. Sequential and overlapping octapeptides corresponding to the MOMP VDIV sequence (residues 288-314) were synthesized on prederivated polypropylene pins and tested individually against a 1:200 dilution of pooled mouse sera for IgG antibody reactivity. The octapeptides tested are provided at the bottom of Figure 2. Each of the mouse strains produced IgG antibodies reactive with VDIV octapeptides containing the LNPTIAG B-cell epitope. Interestingly, five of the six strains immunized with peptide A8-VDIV produced antibodies with enhanced immunoreactivity primarily to those VDIV octapeptides containing the targeted LNPTIAG B-cell epitope.

The immunogenic properties of peptide A8-VDIV in mice were very encouraging and led us to further evaluate the peptides immunogenicity in sub-human primates. Table 2 shows the serum IgG antibody response of three cynomolgus monkeys (705, 752 and 907) after tertiary immunization with peptide A8-VDIV and two control monkeys (842 and 880) that received adjuvant alone. All three immunized monkeys produced significant IgG antibodies to peptide VDIV and *C. trachomatis* serovars D, E, F, G and K. The monkey sera were non-reactive or reacted weakly with the C-complex serovars H, I or J. None of

the immunized monkeys produced IgG antibodies reactive with peptide A-8. The two control monkeys that were immunized with adjuvant only, did not produce IgG antibodies reactive with peptide VDIV or EBs of the different *C. trachomatis* serovars tested.

TABLE 2

Immunogenicity of Peptide A8-VDIV in Cynomolgus Monkeys

		ELISA IgG antibody titer					Peptide pin Mapping	Neutralizing antibody titer (ND_{50})		
		C. trachomatis serovar						*C. trachomatis* serovar		
Monkey number	Peptide VDIV	D	E	F	G	H	response to (LNPTIAG)	D	G	H
705	4096	4096	2048	2048	2048	32	+	256	128	<16
752	1024	2048	512	2048	2048	64	+	128	64	<16
907	512	4096	1024	4096	4096	128	+	256	128	<16
842	<16	<16	<16	<16	<16	<16	-	<16	<16	<16
880	<16	16	<16	16	16	16	-	<16	<16	<16

Monkey sera were further analyzed by pepscan ELISA against sequential and overlapping octapeptides corresponding to the VDIV sequence (Figure 5). All three immunized monkeys produced IgG antibodies that showed strong immunoreactivity against those VDIV octapeptides that contained the targeted LNPTIAG B-cell epitope. These findings clearly showed that immunization with the A8-VDIV peptide was capable of preferentially directing antibody responsiveness to the LNPTIAG epitope contained in the VDIV portion of the oligopeptide immunogen, and this targeted B-cell responsiveness was consistently observed in both mice and primates.

DETERMINATION OF SEROVAR SPECIFICITY AND CROSS-REACTIVITY

To ascertain whether the mouse anti-peptide antibodies reacted with intact chlamydiae, and to determine the serovar specificity of the anti-peptide response, mouse sera were tested by ELISA against formalin-fixed *C. trachomatis* serovars D, E, F, G, H, I, J and K EBs (Fig. 4). The anti-peptide antibodies produced by each mouse strain reacted by ELISA with B-complex (serovars D and E) and Intermediate complex (serovars F, G and K) EBs. These same sera were weakly reactive or non-reactive with the C-complex serovars H, I and J. Results are summarized in Table 3 below. Thus, the *C. trachomatis* serovar specificity of the polyclonal antibodies produced following immunization with peptide A8-VDIV was very similar to that of mAb DIII-A3. The exception was that the sera reacted with serovar K which is non-reactive with mAb DIII-A3.

TABLE 3

The Ability of Peptide A8-VDIV to Prime Mice for an Augmented IgG Response Following Secondary Challenge with Whole C. trachomatis EBs

		ELISA IgG antibody titer							
		Primary immunization				Secondary immunization			
Immunogen			C. trachomatis serovar				C. trachomatis serovar		
Primary	Secondary	Peptide VDIV	D	G	H	Peptide VDIV	D	G	H
PBS	D EB	<16	<16	<16	<16	128	4096	16	<16
A8-VDIV	PBS	1024	4096	1024	16	4096	8192	2048	32
A8-VDIV	D EB	2048	4096	1024	16	16384	32768	16384	512
A8-VDIV	G EB	1024	4096	512	<16	16384	65536	16384	256
A8-VDIV	H EB	1024	4096	512	16	16384	65536	16384	512

TESTING NEUTRALIZING ACTIVITY OF ANTI-A8-VDIV ANTIBODY

To ascertain whether the anti-peptide antibodies generated in the test animal or human patient, in response to the synthetic peptide, were functional neutralizing antibodies, the sera was tested for neutralizing activity using a chlamydial inclusion forming unit (IFU) reduction assay. In this example, the sera from vaccinated mice was tested for neutralizing activity (See Example 6). Neutralization assays were performed with *C. trachomatis* serovars D, G and H, since these three serovars are representative of the three chlamydial serogroups. All six responsive mouse strains had significant serum neutralizing activity against *C. trachomatis* serovars D and G but failed to neutralize the infectivity of serovar H for HaK cells (Fig. 4). The exception was strain B10.A which produced a low neutralizing titer (1:16) against serovar H. Thus, similar to the results found by ELISA using intact *C. trachomatis* 2EBs, the serum neutralizing activity of mouse anti-A8-VDIV was also sub-species specific with only B and Intermediate complete serovars (D and G, respectively) being neutralized. Therefore, it is contemplated that the preferred peptide vaccine of this invention can additionally incorporate the B-cell neutralizing epitope of strains with low neutralizing activity such as strain B10.A.

Sera from monkeys immunized with peptide A8-VDIV (705, 752 and 907) were assayed for their ability to neutralize *C. trachomatis* infectivity *in vitro*. The sera from each of the immunized monkeys had significant neutralizing activity against *C. trachomatis* serovars D and G but were incapable of neutralizing the infectivity of serovar H (Figure 6). Thus, primates immunized with peptide A8-VDIV produced neutralizing antibodies having a *C. trachomatis* sub-species specificity. These findings were consistent with those found in

the mouse and clearly demonstrated the ability of peptide A8-VDIV to evoke broadly cross-reactive neutralizing antibodies in both mice and primates.

Summaries of the neutralization studies are provided for mice and monkeys in Tables 1 and 2 respectively. These findings demonstrated, as a whole, that mouse and monkey anti-A8-VDIV antibodies are capable of neutralizing the infectivity of chlamydiae *in vitro*.

COMPARISON OF A8-VDIV PEPTIDE VACCINE WITH A8-VDI(A) VACCINE

Previously, a peptide vaccine A8-VDI(A) was prepared that combined the conserved T-helper cell epitope of this invention with another B cell neutralizing epitope. The following study compared the two vaccines for its ability to induce a protective response against a number of *C. trachomatis* serovars in primates. Advantageously, the combination of the conserved T-helper epitope with the VDIV-derived neutralizing epitope provided a broad protective response to a number of serovars as compared to A8-VDI(A) and as compared with other combinations of epitopes.

Monkeys were immunized three times intramuscularly with 1 mg of peptide. Animals were bled 14 days following the third immunization and their sera was tested by ELISA for IgG antibodies reactive against multiple *C. trachomatis* serovars. Results are provided in Figure 7. Solid bars represent absorbance values (A_{405}) of pre-immune sera. The stippled bars are absorbance values of sera after immunization with the synthetic peptides. Monkeys immunized with peptide A8-VDI produce antibodies against only serovar A. In contrast, monkeys immunized with peptide A8-VDIV produced IgG antibodies reactive with the majority of the serovars. Most significantly, monkey anti-A8-VDIV antibodies reacted strongly against *C. trachomatis* serovars D, E, F, and G; the most important serovars in terms of *C. trachomatis* caused sexually transmitted diseases (STD). The anti-A8-VDIV sera neutralized chlamydial infectivity *in vitro* with a similar specificity as that shown by ELISA. Thus, the A8-VDIV peptide has considerable potential as a vaccine to prevent infection in humans by *C. trachomatis* because it is capable of evoking broadly cross reactive neutralizing antibodies against multiple serovars.

As provided in the invention disclosed above, this invention has particular utility against the major serovars associated with STDs in this country. Advantageously, and unlike other C. trachomatis vaccines disclosed thus far, the synthetic peptide vaccine of this invention has proved useful against challenges of *C. trachomatis* in mice and primates. The results of studies with this vaccine indicated that immunization with the peptide effectively targeted the production of high titer antibodies against the B-cell portion of the peptide.

These anti-peptide antibodies were preferentially directed at the antigenically common B-cell epitope LNPTIAG within the VDIV sequence, recognized this epitope in its native configuration, and were functional antibodies capable of neutralizing the infectivity of those *C. trachomatis* serovars that are epidemiologically important as causative agents of chlamydial STDs.

Synthetic peptide and recombinant subunit immunogens tend to be poorly immunogenic, antibodies produced against them may not recognize the targeted B-cell epitope within the peptide's primary sequence, and the anti-peptide antibodies may fail to react with the targeted B-cell epitope in its native configuration on the pathogens' surface. In addition, the number of T-helper cell epitopes that can be incorporated into synthetic peptide immunogens is restricted. This too can compromise the immunogenicity of the peptide within the general population since HLA class II diversity is known to influence the recognition of T-cell antigens (Schwartz, R.H. *Curr. Top. Microbiol. Immunol.* 130:79, 1986). The results of these studies in mice indicated that six of eight H-2 congenic mouse strains that were immunized with peptide A8-VDIV produced IgG antibodies reactive with the B-cell portion of the peptide and these findings are consistent with the hypothesis that the T_H-cell epitope(s) contained in the A8 sequence is recognized by multiple MHC class II haplotypes. Two of the responding strains of mice, C57BL/10 and B10.M also produced antibodies following immunization with peptide VDIV alone; indicating that in addition to B-cell epitopes, the VDIV sequence also contained a T_H-cell epitope whose recognition is restricted by H-2^b and H-2^f haplotypes. Thus, peptide A8-VDIV contains at least two distinct epitopes that elicit functional T_H activity. The combination of these two sites enhance the possibility of the peptide being generally recognized in the heterogenous human population. Consistent with this hypothesis is the finding that all three cynomolgus monkeys immunized with peptide A8-VDIV produced IgG antibodies against the targeted LNPTIAG B-cell epitope. We have recently immunized four other cynomolgus monkeys with peptide A8-VDIV as part of a separate study to evaluate the protective efficacy of the peptide immunogen. All four of the vaccinated monkeys produced IgG antibodies against the LNPTIAG neutralizing epitope further supporting the potential of the peptide immunogen to be generally recognized.

Surprisingly, our findings suggest that peptide A8-VDIV does not share the unfavorable immunological characteristics that have been commonly associated with synthetic peptide immunogens. It is not understood why immunization with peptide A8-VDIV was so effective in evoking antibody responsiveness to the LNPTIAG epitope within

the VDIV sequence. It is possible, because of the size of the VDIV sequence (17 amino acids) incorporated in the A8-VDIV peptide, that the peptide maintained structural elements important in determining its immunogenicity. Our findings convincingly demonstrate that the A8-VDIV peptide is a very effective immunogen that is capable of preferentially evoking antibody responsiveness to the antigenically common LNPTIAG neutralizing epitope contained within the VDIV sequence.

An unexpected finding of this work was that both the mouse and monkey anti-A8-VDIV antisera were reactive against serovar K (Fig. 3 and Table I). These data are not consistent with the immunoreactivity of mAb DIII-A3 which does not react with the MOMP of serovar K by western blotting (see Zhang et al., *Infect. Immun.*, (1989) supra). The lack of immunoreactivity of mAb DIII-A3 with serovar K can be explained by the threonine for alanine substitution at position 303 of the LNPTIA(T)G sequence. Unlike mAb DIII-A3 which reacts with a single epitope, polyclonal anti-A8-VDIV antibodies reacted with multiple B-cell epitopes contained in the VDIV sequence. This is evident from the pepscan analysis of the polyclonal anti-A8-VDIV response (Figs. 2 and 5) which demonstrated in addition to octapeptides containing the LNPTIAG epitope, both mice and monkeys produced antibodies reactive with other octapeptides corresponding to VDIV sequences. Although it is difficult to determine which epitope(s) is important for the reactivity of the anti-peptide antibodies with serovar K, an epitope(s) contained within the ^{296}TTLNPTI^{302} sequence is a likely possibility. This sequence is present in VDIV of serovar K and the anti-peptide antibodies were immunoreactive with octapeptides containing the TTLNPTI sequence by pepscan analysis. The TTLNPTI sequence is also common to the C-complex serovars H, I and J, however, like the LNPTIAG epitope, it is apparently not accessible to antibody on the native EB surfaces of these serovars. Nonetheless, the fact that immunization with peptide produced antibodies reactive with serovar K is clearly advantageous because it increases the number of *C. trachomatis* serovars to which peptide A8-VDIV can evoke neutralizing antibodies.

A more precise evaluation of the peptides vaccine potential will require phase I vaccine studies in humans to define its immunogenicity and toxicity. In this context, we observed that none of the primates immunized with the oligopeptide developed clinical signs of allergenic or toxigenic reactivities suggesting that the peptide could be safely administered to humans.

Particular embodiments of the invention will be discussed in detail and reference will be made to possible variations within the scope of the invention. There are a variety of

alternative techniques and procedures available to those of skill in the art which would similarly permit one to successfully perform the intended invention.

Example 1

Construction of a Synthetic Vaccine Containing a Conserved B cell Neutralizing Epitopes and a conserved T cell Epitope

Peptides VDIV, and A8-VDIV were synthesized using an automated peptide synthesizer (Model 431A Synthesizer, Applied Biosystems, Inc., Foster City, CA) as described previously by Su et al., *supra*. Peptides were purified by reverse phase HPLC on a C18 column (Beckman Instruments, Inc., Fullerton, CA). The accuracy of the synthesis reaction was defined by amino acid sequencing. Peptide VDIV corresponding to serovar B MOMP residues 293-309 (FDVTTLNPTIAGAGDVK) and containing the sequence invariant LNPTIAG septmeric epitope that is recognized by the neutralizing mAb DIII-A3. Peptide A8 corresponds to serovar A MOMP residues 106-130 (ALNIWDRFDVFCTLGATTGYLKGNS) which contains a MOMP T_H-cell epitope that effectively primes mice to produce an anamnestic IgG response specific to MOMP following secondary immunization with the native protein. Peptide A8-VDIV consists of peptide A8 and VDIV colinearly synthesized with the A8 sequence at its N-terminus and the VDIV sequence at its carboxyl terminal end.

Example 2

Vaccination of Mice with Synthetic Peptide

C57BL/10SnJ (H-2^b), B10.A/SgSnJ (H-2^a), B10.D2/oSnJ (H-2^d), B10.M/Sn (H-2^f), B10.WB/Sn (H-2^{ja}), B10.Br/SgSnJ (H-2^k), B10.PL(73NS)/Sn (H-2^u) and B10.SM(70NS)/Sn (H-2^v) mice were purchased from Jackson Laboratory (Bar Harbor, ME). Both sexes of mice at 8-12 weeks of age were used for experimentation. Groups of five mice were immunized by intraperitoneal injection of 50 μg of peptide A8-VDIV or peptide VDIV alone emulsified in complete Freund's adjuvant (CFA) and boosted once three weeks later with the same dose of peptide in incomplete Freund's adjuvant (IFA). Mice were bled two weeks after the secondary immunization.

Example 3

Vaccination of Primates with Synthetic Peptide

Cynomolgus (*Macaca fasicularis*) monkeys of Mauritius Island origin were used in these studies. Monkeys were part of a fully condition colony that had been stable for over two years. All examinations of experimental monkeys were conducted using ketamine

hydrochloride sedation. The work was conducted in full compliance with the "Guide for Case and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences National Research Council," as well as all applicable federal laws and regulations. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). Three cynomolgus monkeys (705, 752, and 907) were immunized intramuscularly with 1 mg of peptide A8-VDIV emulsified with Ribi adjuvant (MPL+TDM+CWS emulsion, Ribi Immunochem Research, Inc., Hamilton, MT). Two control monkeys (842 and 880) were immunized with adjuvant alone. Immunization was repeated twice with 500 µg of peptide and adjuvant at four week intervals. Monkeys were bled two weeks after the third immunization.

Example 4

Serologic Evaluation of Antibody Response

C. trachomatis serovars D (UW-3/Cx), E (Bour), F (IC-Cal-13), G (UW-57/Cx), H (UW-4/Cx), I (UW-12/Ur), J (WU-36/Cx), and K (UW-31/Cx) were grown in HeLa 229 cells and chlamydial elementary bodies (EBs) were purified from infected cells by density gradient centrifugation as previously described by Caldwell et al., *supra*.

Serum antibody responses were assayed by enzyme linked immunoabsorbent assay (ELISA) following previously described methods (Su et al., (1990) *supra*). Briefly, microtiter plates (Immunolon 2; Dynatech Laboratories, Inc. Alexandria, VA) were coated overnight at 4oC with 100 µl of synthetic peptide (5 µg/ml), purified MOMP (0.5 µg/ml), or formalin killed *C. trachomatis* EBs (10 µg/ml) in 0.05 M Tris buffer (pH 7.5) containing 0.15 M NaCl. Serial twofold diluted mouse or monkey sera were tested in duplicate. Mouse and monkey IgG were detected using anti-mouse and anti-human IgG alkaline phosphatase conjugate respectively (γ chain specific, Zymed Laboratories, Inc., San Francisco, CA) followed by substrate (5 mg p-nitrophenyl phosphate in 10 ml. of 0.1 M 2,2 amino-2-methyl-1,3-propandiol, pH 10.3). Absorbance at 405 nm was measured with an ELISA reader (Bio-Rad Laboratories, Richmond, CA). Pooled pre-immune sera was used as the negative controls and ELISA titers were expressed as the highest serum dilution giving an absorbance of 0.3 OD units.

Example 5

Defining Linear Epitopes Associated with the Immune Response

Linear epitopes from MOMP that bound serum antibody from vaccinated mice and monkeys were identified by pepscan assay as described by Geysen, et al. (*J. Immunol.*

Methods 102:259, 1989). Sequential and overlapping octapeptides corresponding to serovar B MOMP VDIV (residues 288-314) were synthesized on prederivatized pins using a commercially available kit (Epitope Scanning Kit, Cambridge Research Biochemicals, Inc., Wilmington, DE) following the instructions of the manufacturer. Reactivity of mouse and monkey IgG antibodies to the solid phase octapeptides were determined by ELISA using the same anti-mouse or human IgG alkaline phosphatase conjugate as described above for the ELISA assay.

Example 6

In vitro neutralization of *C. trachomatis* infectivity.

In vitro neutralization of *C. trachomatis* infectivity by mouse or monkey anti-peptide antibodies was assayed on Syrian hamster kidney (HaK) cells grown in 96-well microtiter plates (Linbro, 96 flat-bottomed wells, Flow Laboratories, Inc., McLean, VA) as described previously (Su, H. and H.D. Caldwell., *J. Exp. Med.* 175:227 (1992). Briefly, 10^5 HaK cells were seeded in 96-well plates 24 hours prior to the neutralization assay. *C. trachomatis* serovars D, G, and H were diluted in 250 mM sucrose, 10 mM sodium phosphate, 5 mM L-glutamic acid, pH 7.2 (SPG) to give a final concentration of 3×10^5 - 3×10^6 inclusion-forming units (IFUs)/ml. Two fold dilutions of pooled mouse sera or individual monkey sera were mixed with an equal volume of chlamydiae and incubated at 37°C for 30 minutes. The mixtures (50µl/well) were inoculated in triplicate onto HaK cell monolayers. After 2 hours incubation at 37°C, the inocula were removed, the monolayers washed and refed with media. The monolayers were fixed with methanol after incubation at 37°C for 48 hours and IFUs were identified by indirect fluorescent antibody staining. Serum neutralizing titers were expressed as percent reduction in chlamydial IFUs and were calculated as follows: [(IFUs control sera - IFUs experimental sera)/IFUs control sera] X 100.

While particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art that these embodiments are exemplary rather than limiting, and the true scope of the invention is that defined in the following claims.

SEQUENCE LISTING

1) GENERAL INFORMATION:

(i) APPLICANT: The Government of the United States of America as represented by the Secretary of the Department of Health and Human Services

(ii) TITLE OF INVENTION: SYNTHETIC PEPTIDE VACCINE FOR CHLAMYDIA TRACHOMATIS

(iii) NUMBER OF SEQUENCES: 3

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/947,671 US
(B) FILING DATE: 18 SEP 93

2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala
1 5 10 15

Thr Thr Gly Tyr Leu Lys Gly Asn Ser
20 25

2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Phe Asp Val Thr Thr Leu Asn Pro Thr Ile Ala Gly Ala Gly Asp Val
1               5                  10                  15

Lys
```

2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala
1               5                  10                  15

Thr Thr Gly Tyr Leu Lys Gly Asn Ser Phe Asp Val Thr Thr Leu Asn
            20                  25                  30

Pro Thr Ile Ala Gly Ala Gly Asp Val Lys
        35                  40
```

WHAT IS CLAIMED IS:

1. A synthetic peptide capable of producing an immunological response to *C. trachomatis* in a vertebrate, comprising:

a conserved T-helper cell stimulating epitope from the major outer membrane protein of *C. trachomatis*; and

a serovar conserved B-cell neutralizing antibody stimulating epitope from the major outer membrane protein of *C. trachomatis*.

2. The synthetic peptide of Claim 1, wherein said T-helper cell stimulating epitope and said B-cell neutralizing antibody stimulating epitope are colinear.

3. The synthetic peptide of Claim 1, wherein said synthetic peptide contains the sequence identified as SEQ ID NO:3.

4. The synthetic peptide of Claim 1, wherein said T-helper cell stimulating epitope has a sequence located within SEQ ID NO: 1.

5. The synthetic peptide of Claim 1, wherein said B-cell neutralizing antibody stimulating epitope has a sequence located within SEQ ID NO: 2.

6. The synthetic peptide of either Claim 4 or 5, wherein said T-helper stimulating epitope has a sequence located within SEQ ID NO:1 and said B-cell neutralizing antibody stimulating epitope has a sequence located within SEQ ID NO:2.

7. The synthetic peptide of Claim 6, wherein said T-helper stimulating epitope is on the N-terminus side of said B-cell neutralizing antibody stimulating epitope.

8. The synthetic peptide of Claim 6, additionally comprising a linker peptide sequence separating the sequence of SEQ ID NO:1 from the sequence of SEQ ID NO:2.

9. The synthetic peptide of Claim 6, wherein said peptide additionally comprises at least one species specific B-cell neutralizing antibody stimulating epitope.

10. The synthetic peptide of Claim 7, wherein said synthetic peptide additionally comprises a known T-helper stimulating epitope from a protein other than the major outer membrane protein of *C. trachomatis*.

11. Use of a synthetic peptide according to Claim 1 in the preparation of a vaccine for *C. trachomatis*.

12. The use of Claim 11, wherein said conserved T-helper cell stimulating epitope is an epitope having a sequence located within SEQ ID NO:1 and said serovar conserved B-cell neutralizing antibody stimulating epitope is an epitope having a sequence located within SEQ ID NO:2.

13. An immunoassay for detecting the presence of antibody to *C. trachomatis* in a sample, comprising a synthetic peptide according to Claim 1 and means for detecting antibody bound to said peptide.

14. A method for inducing a protective immune response to *C. trachomatis* in a vertebrate comprising introducing a synthetic peptide according to Claim 1 into said vertebrate, said peptide being introduced along with a pharmaceutically acceptable buffer.

15. The method of Claim 14, additionally comprising, after the introduction step, the step of determining the presence of neutralizing antibody to *C. trachomatis* in said vertebrate

16. The method of Claim 14, wherein the introducing step comprises injecting said synthetic peptide into said vertebrate intramuscularly.

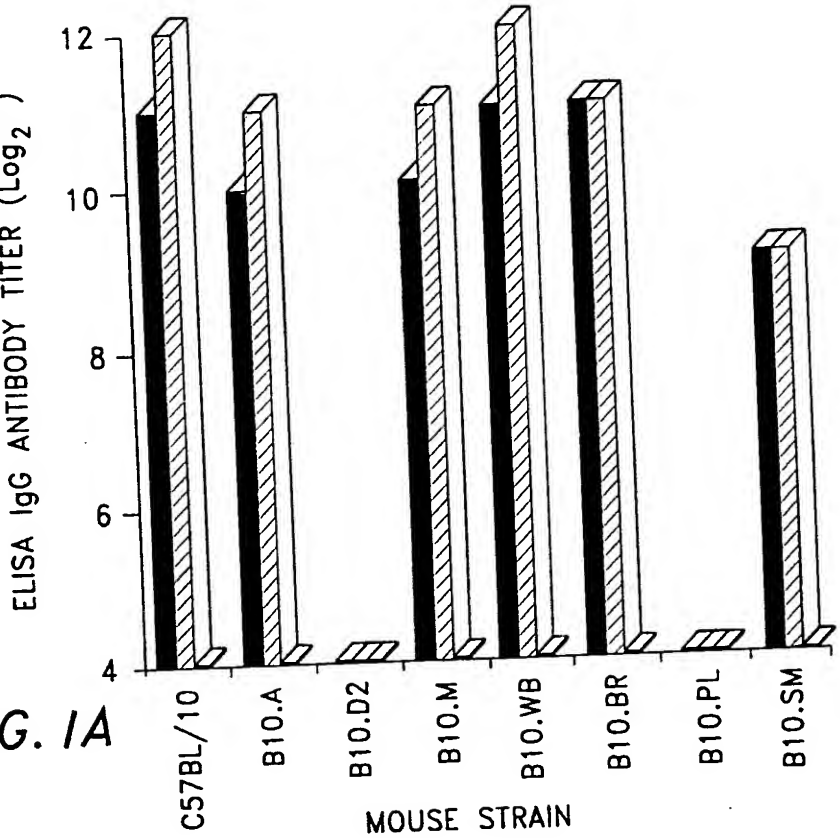

ELISA IgG ANTIBODY TITER (Log_2^{-1})
12
10
8
6
4
C57BL/10
B10.A
B10.D2
B10.M
B10.WB
B10.BR
B10.PL
B10.SM
MOUSE STRAIN

FIG. 1A

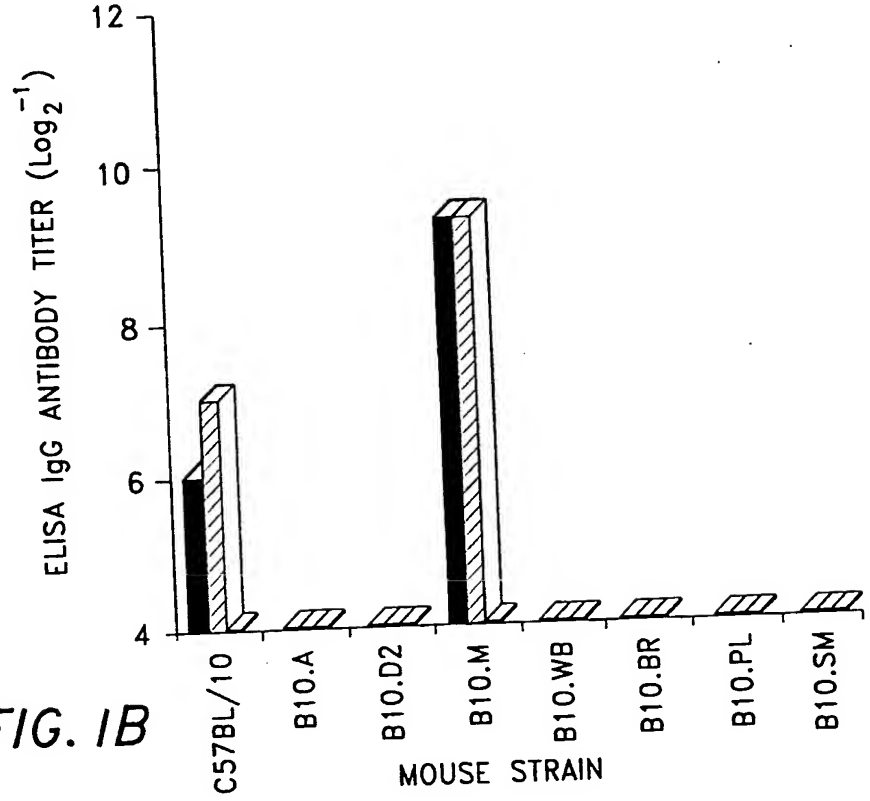
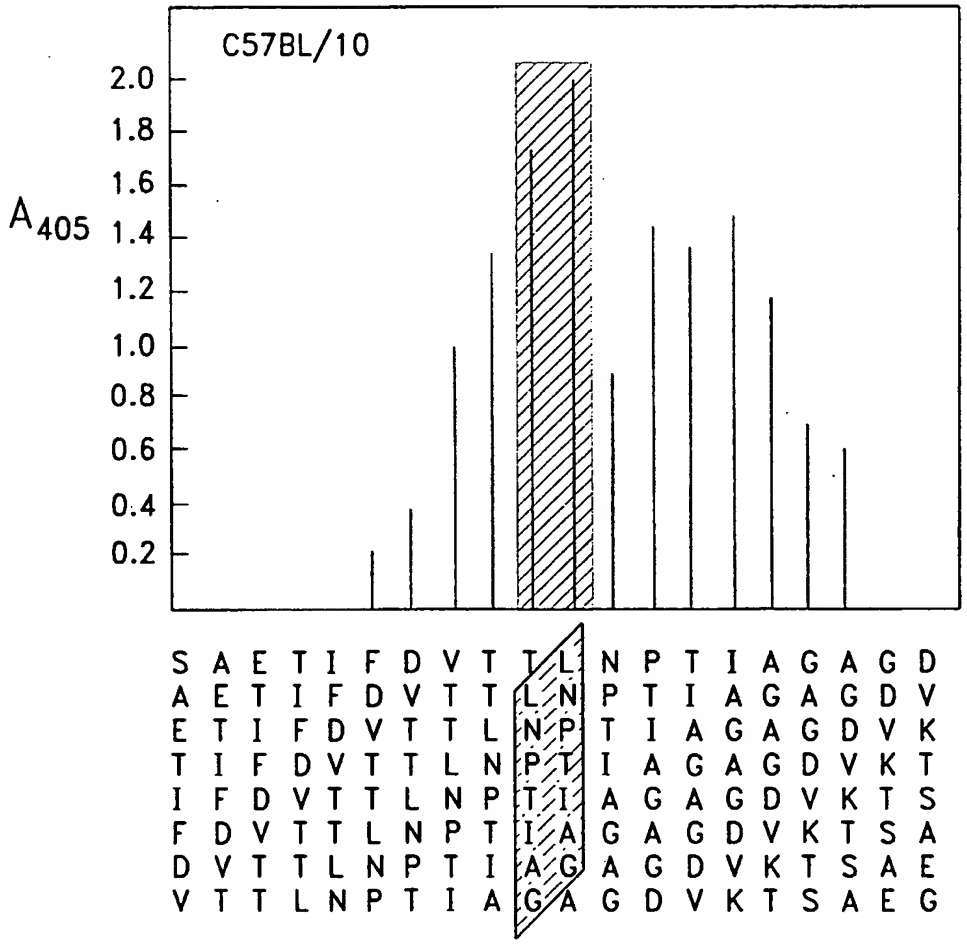

ELISA IgG ANTIBODY TITER (Log_2^{-1})
12
10
8
6
4
C57BL/10
B10.A
B10.D2
B10.M
B10.WB
B10.BR
B10.PL
B10.SM
MOUSE STRAIN

FIG. 1B

FIG. 2A



VDIV (288–314)

FIG. 2B

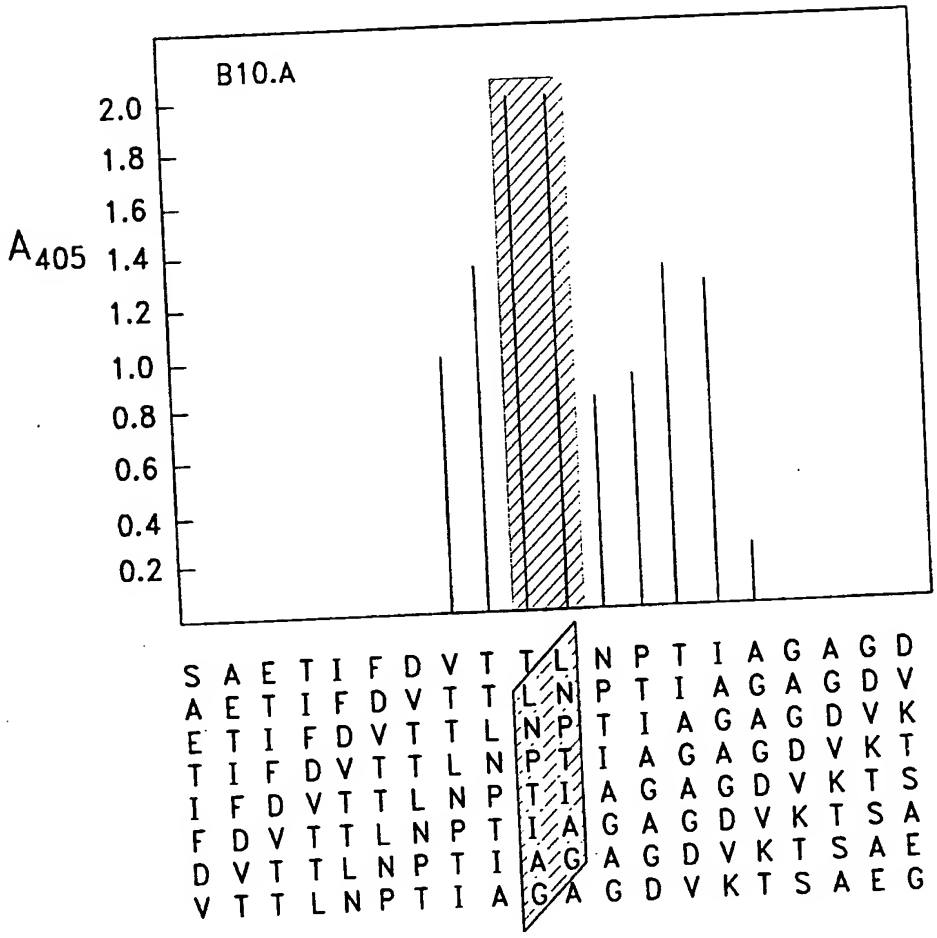
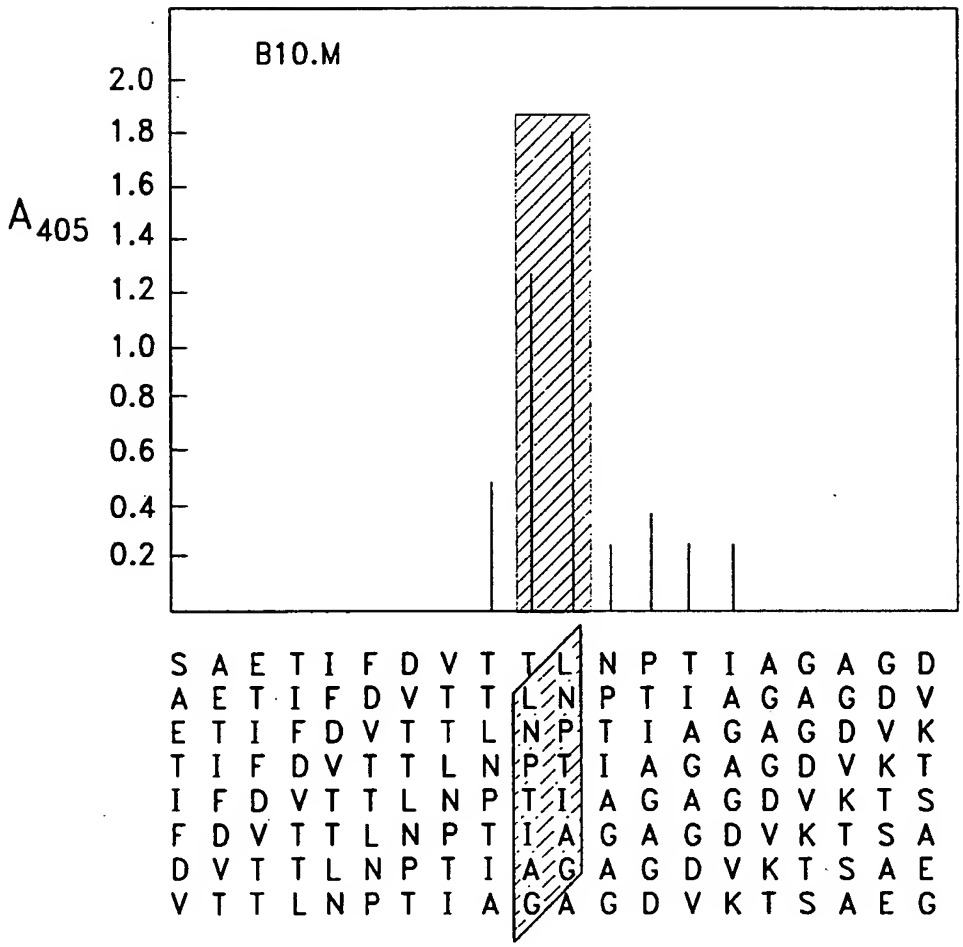
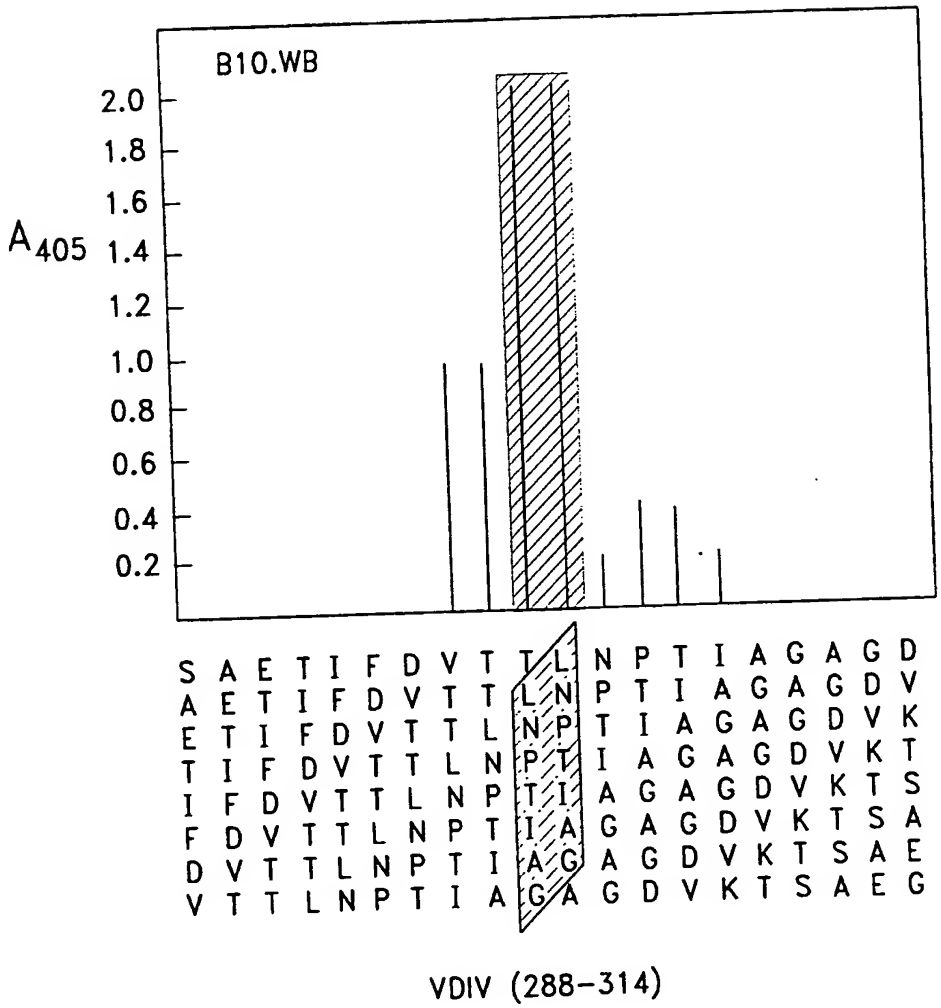

B10.A
A_{405}
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
S A E T I F D V T T L N P T I A G A G D
A E T I F D V T T L N P T I A G A G D V
E T I F D V T T L N P T I A G A G D V K
T I F D V T T L N P T I A G A G D V K T
I F D V T T L N P T I A G A G D V K T S
F D V T T L N P T I A G A G D V K T S A
D V T T L N P T I A G A G D V K T S A E
V T T L N P T I A G A G D V K T S A E G
VDIV (288-314)

FIG. 2C



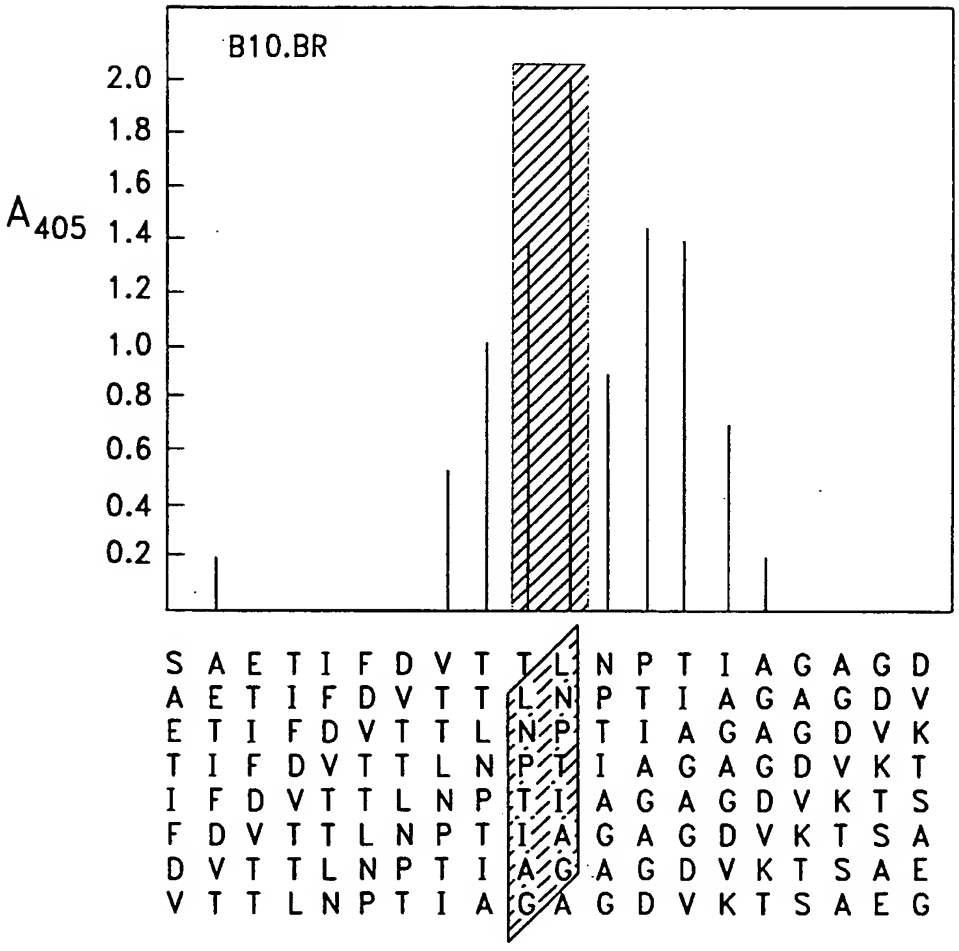
VDIV (288–314)

FIG. 2D



VDIV (288–314)

FIG. 2E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A_{405}
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1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
S A E T I F D V T T L N P T I A G A G D
A E T I F D V T T L N P T I A G A G D V
E T I F D V T T L N P T I A G A G D V K
T I F D V T T L N P T I A G A G D V K T
I F D V T T L N P T I A G A G D V K T S
F D V T T L N P T I A G A G D V K T S A
D V T T L N P T I A G A G D V K T S A E
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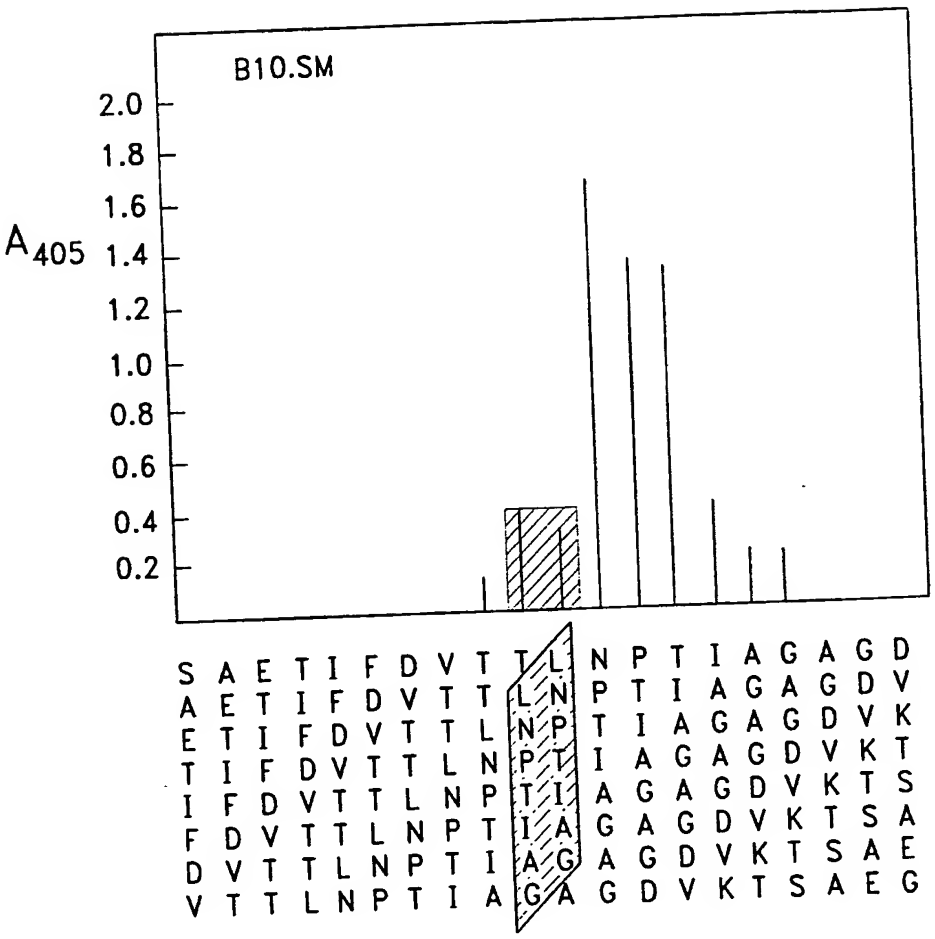
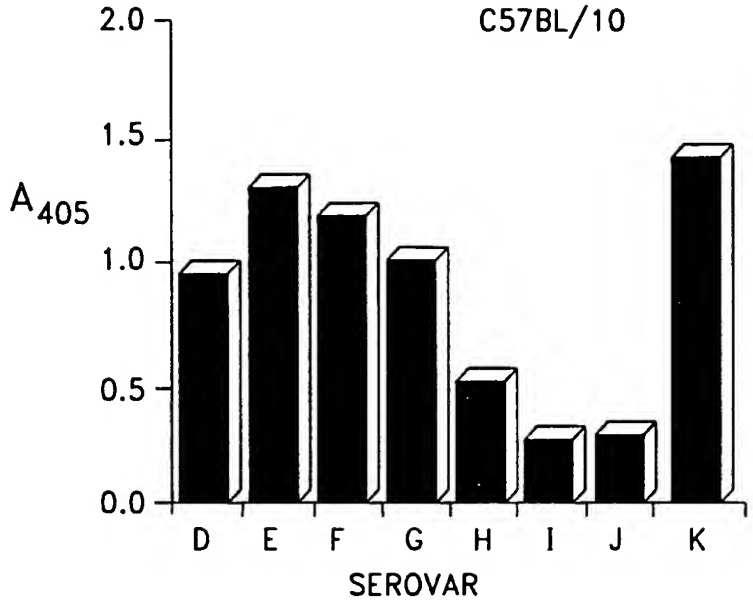
VDIV (288–314)

FIG. 2F



VDIV (288–314)

FIG. 3A


C57BL/10
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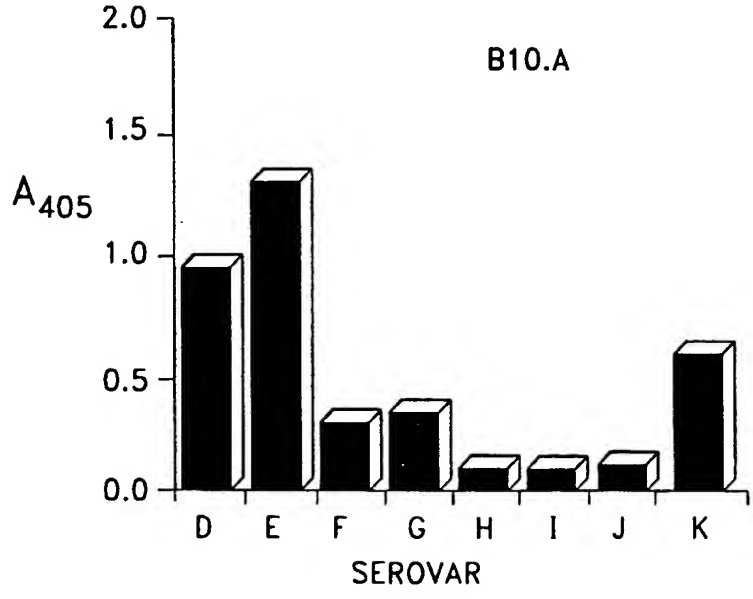
1.0
0.5
0.0
A_{405}
D E F G H I J K
SEROVAR

FIG. 3B


B10.A
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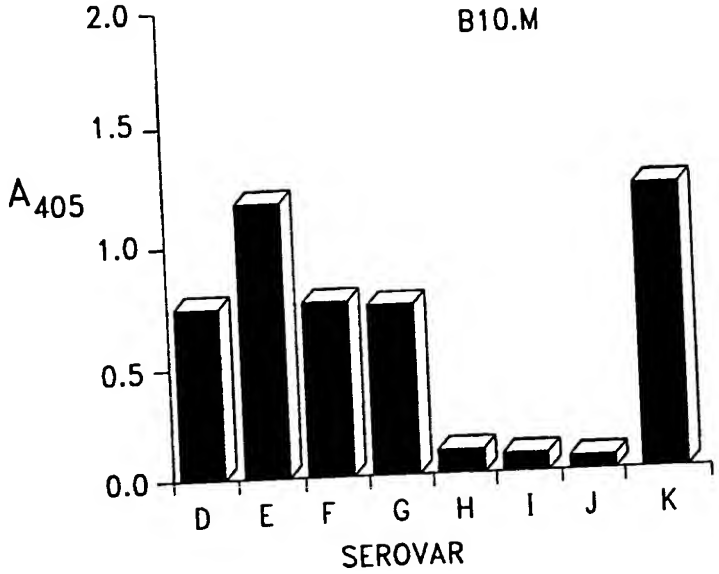
1.0
0.5
0.0
A_{405}
D E F G H I J K
SEROVAR

FIG. 3C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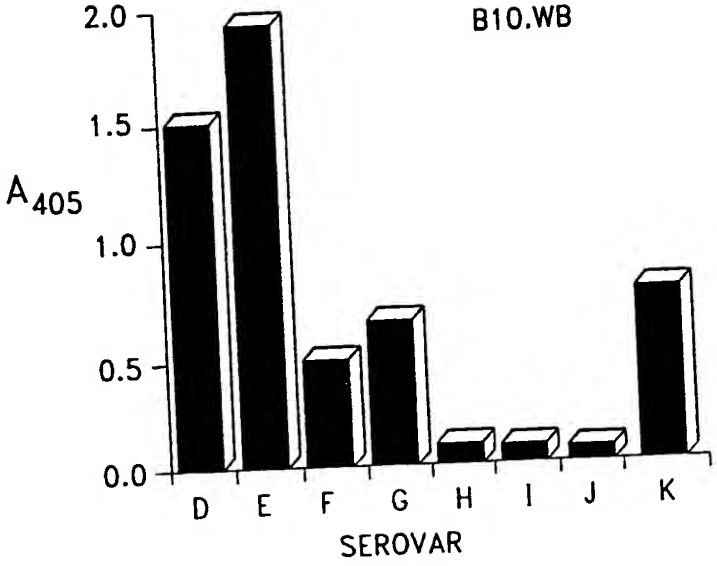
A_{405}
1.0
0.5
0.0
D E F G H I J K
SEROVAR

FIG. 3D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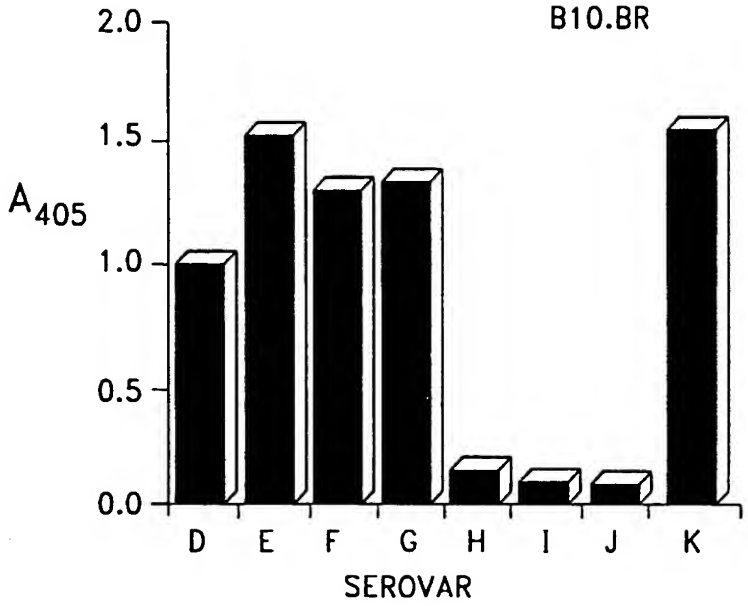
A_{405}
1.0
0.5
0.0
D E F G H I J K
SEROVAR

FIG. 3E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0.5
0.0
A_{405}
D E F G H I J K
SEROVAR

FIG. 3F

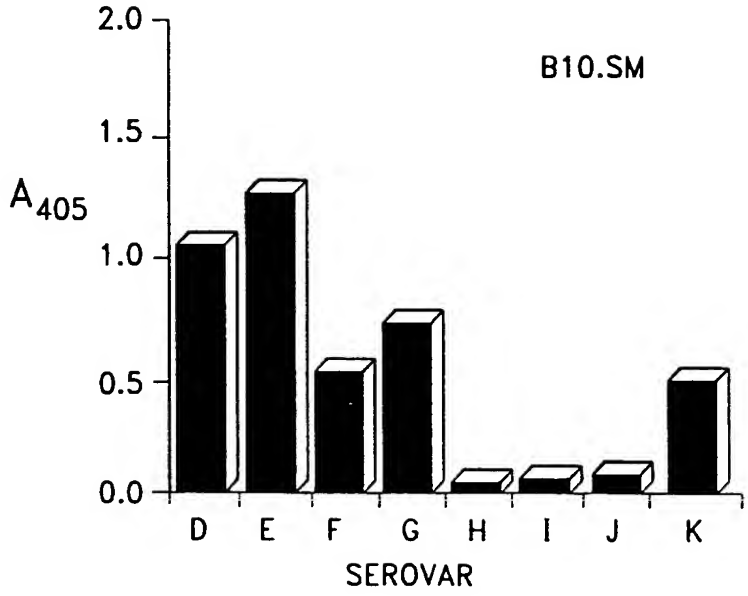
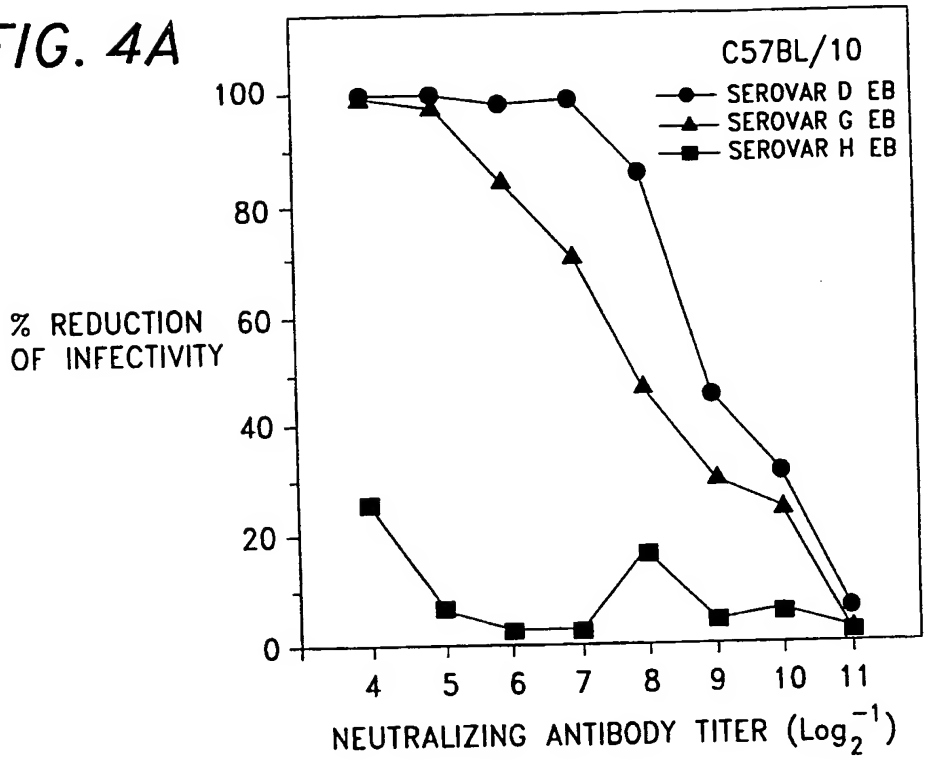

B10.SM
2.0
1.5
1.0
0.5
0.0
A_{405}
D E F G H I J K
SEROVAR

FIG. 4A


C57BL/10
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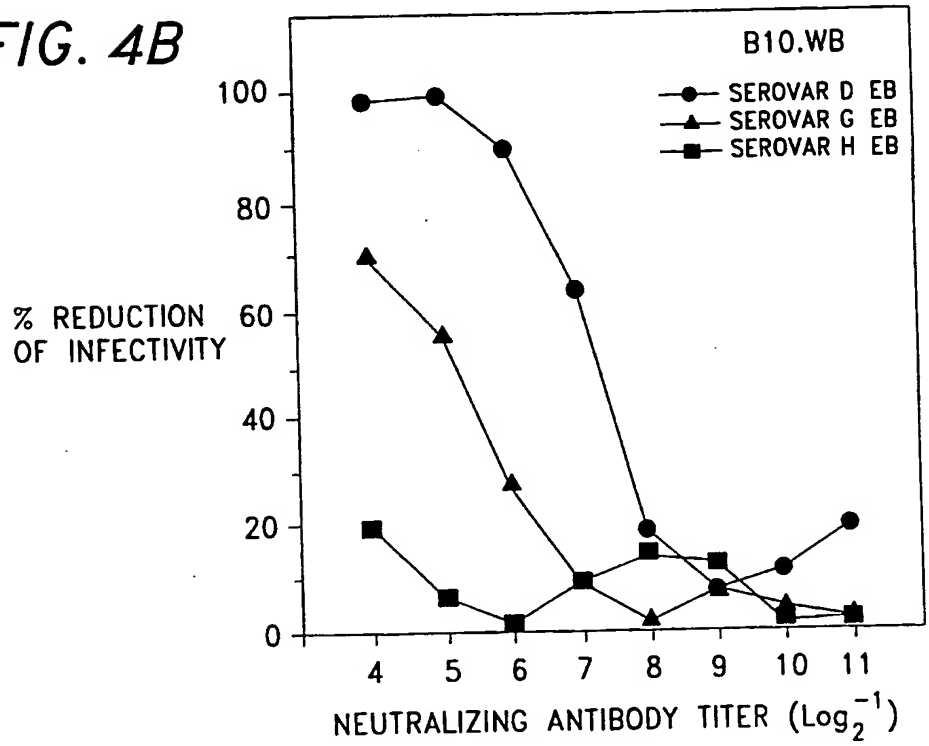
SEROVAR G EB
SEROVAR H EB
% REDUCTION OF INFECTIVITY
100
80
60
40
20
0
4
5
6
7
8
9
10
11
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 4B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SEROVAR G EB
SEROVAR H EB
% REDUCTION OF INFECTIVITY
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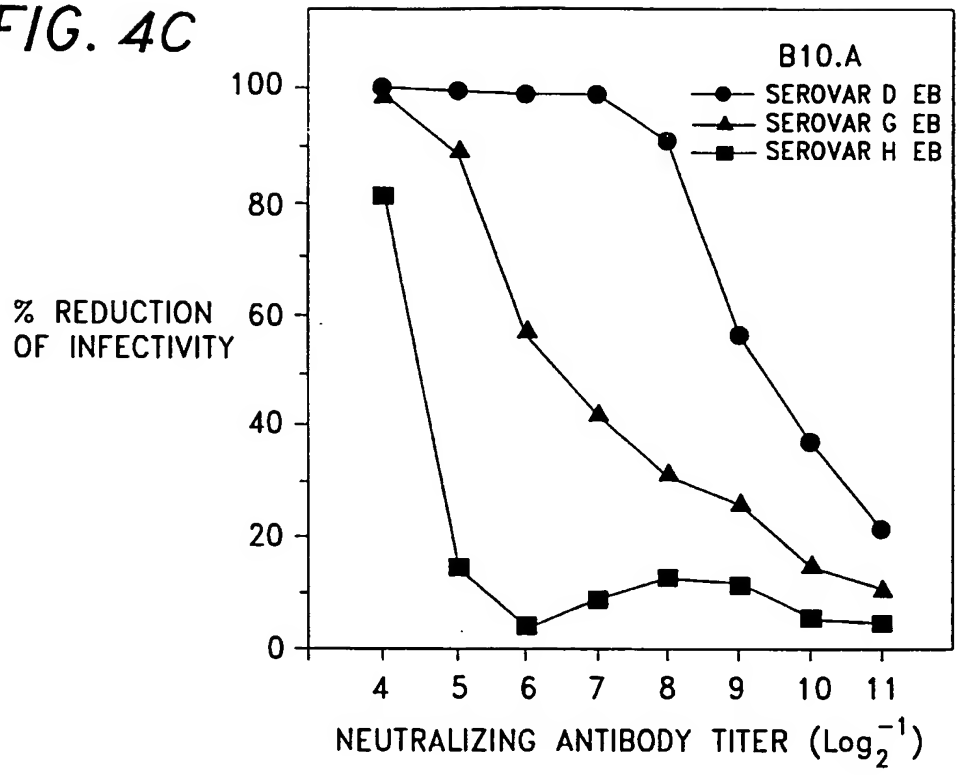
7
8
9
10
11
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 4C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SEROVAR D EB
SEROVAR G EB
SEROVAR H EB
% REDUCTION OF INFECTIVITY
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60
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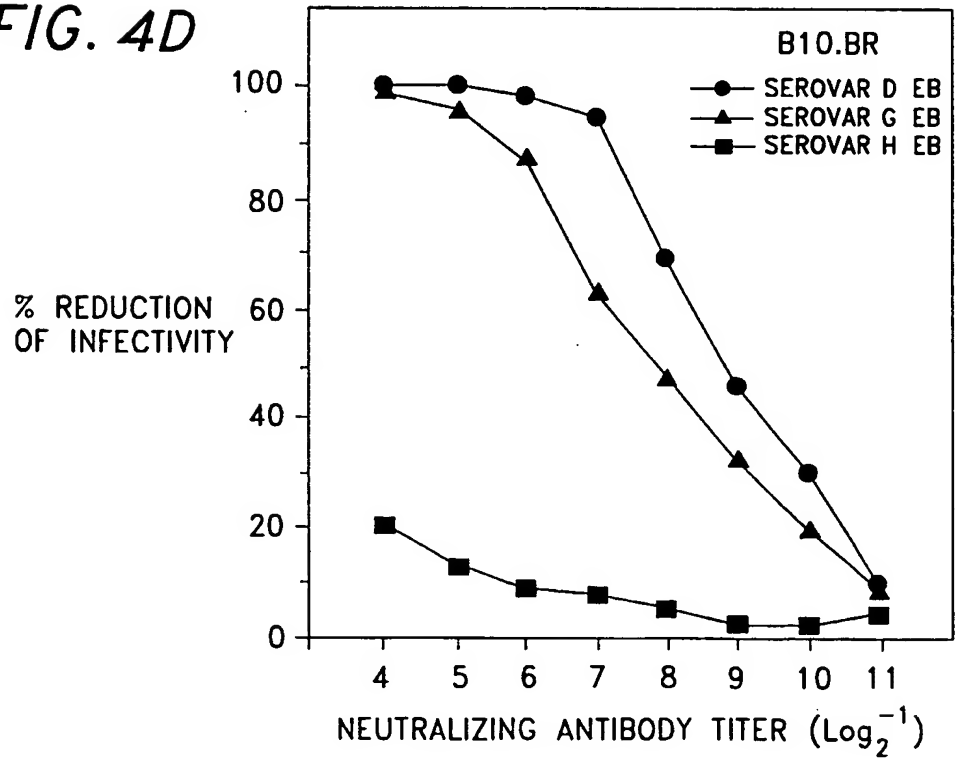
6
7
8
9
10
11
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 4D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SEROVAR G EB
SEROVAR H EB
% REDUCTION OF INFECTIVITY
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80
60
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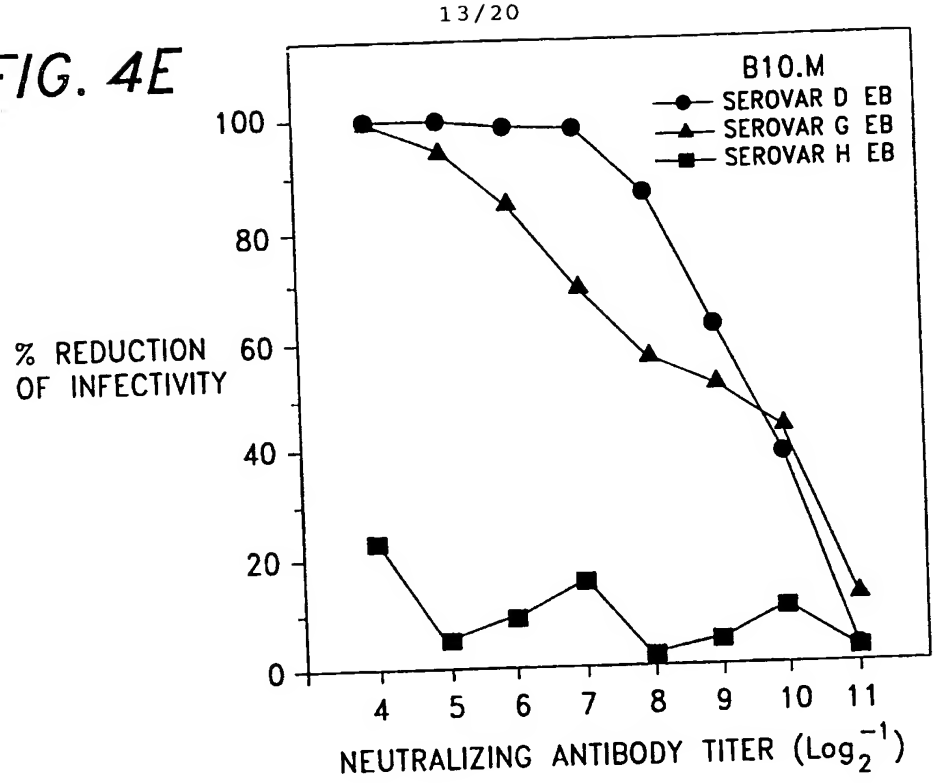
20
0
4
5
6
7
8
9
10
11
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 4E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SEROVAR G EB
SEROVAR H EB
% REDUCTION OF INFECTIVITY
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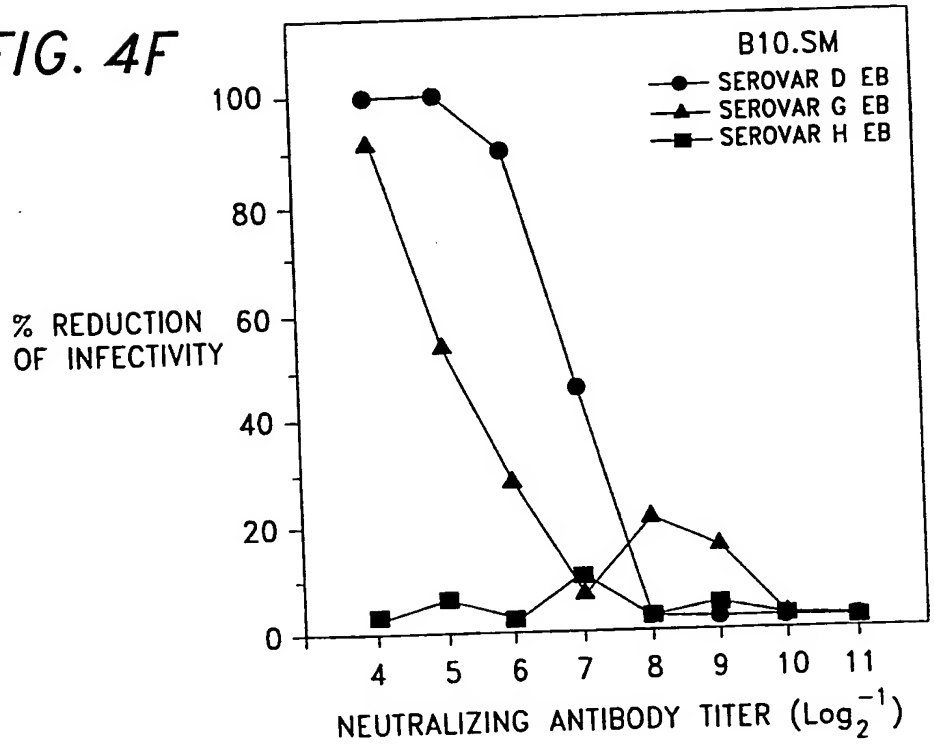
6
7
8
9
10
11
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 4F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% REDUCTION OF INFECTIVITY
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80
60
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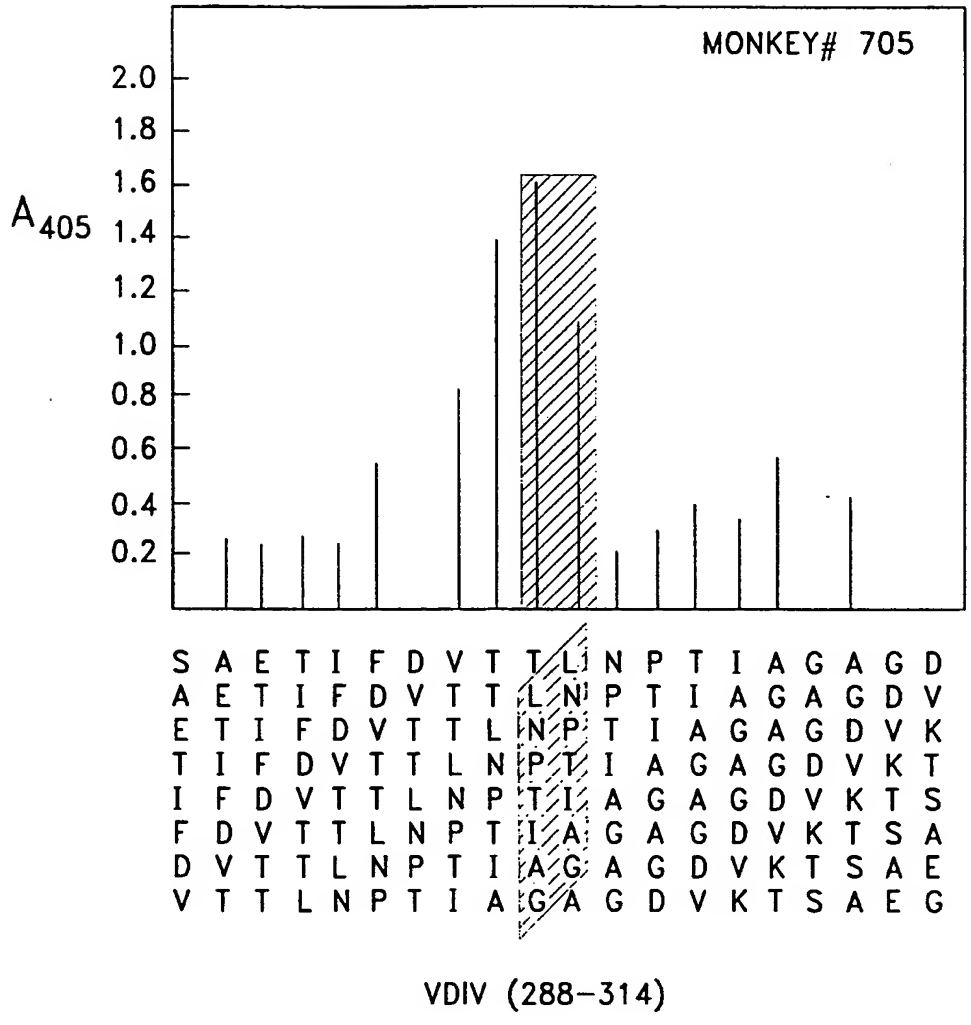
8
9
10
11
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 5A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1.6
1.4
1.2
1.0
0.8
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0.2
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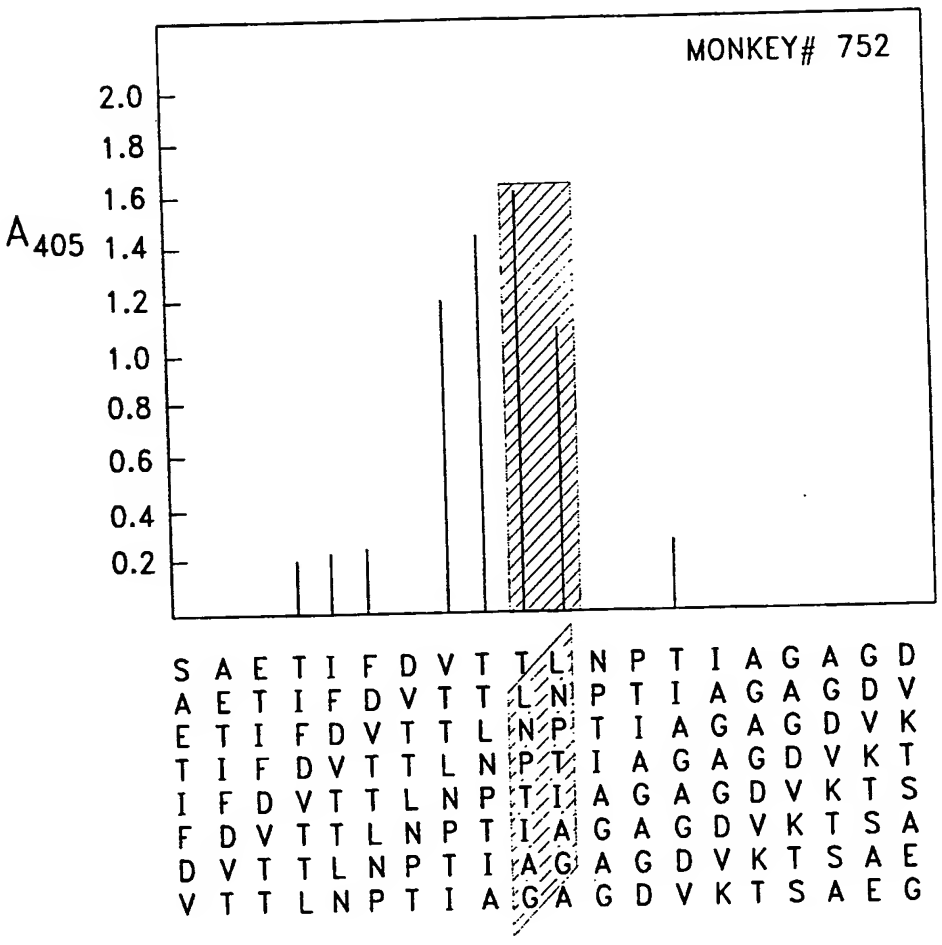
A E T I F D V T T L N P T I A G A G D V
E T I F D V T T L N P T I A G A G D V K
T I F D V T T L N P T I A G A G D V K T
I F D V T T L N P T I A G A G D V K T S
F D V T T L N P T I A G A G D V K T S A
D V T T L N P T I A G A G D V K T S A E
V T T L N P T I A G A G D V K T S A E G
VDIV (288–314)

FIG. 5B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A_{405}
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1.0
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0.6
0.4
0.2
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A E T I F D V T T L N P T I A G A G D V
E T I F D V T T L N P T I A G A G D V K
T I F D V T T L N P T I A G A G D V K T
I F D V T T L N P T I A G A G D V K T S
F D V T T L N P T I A G A G D V K T S A
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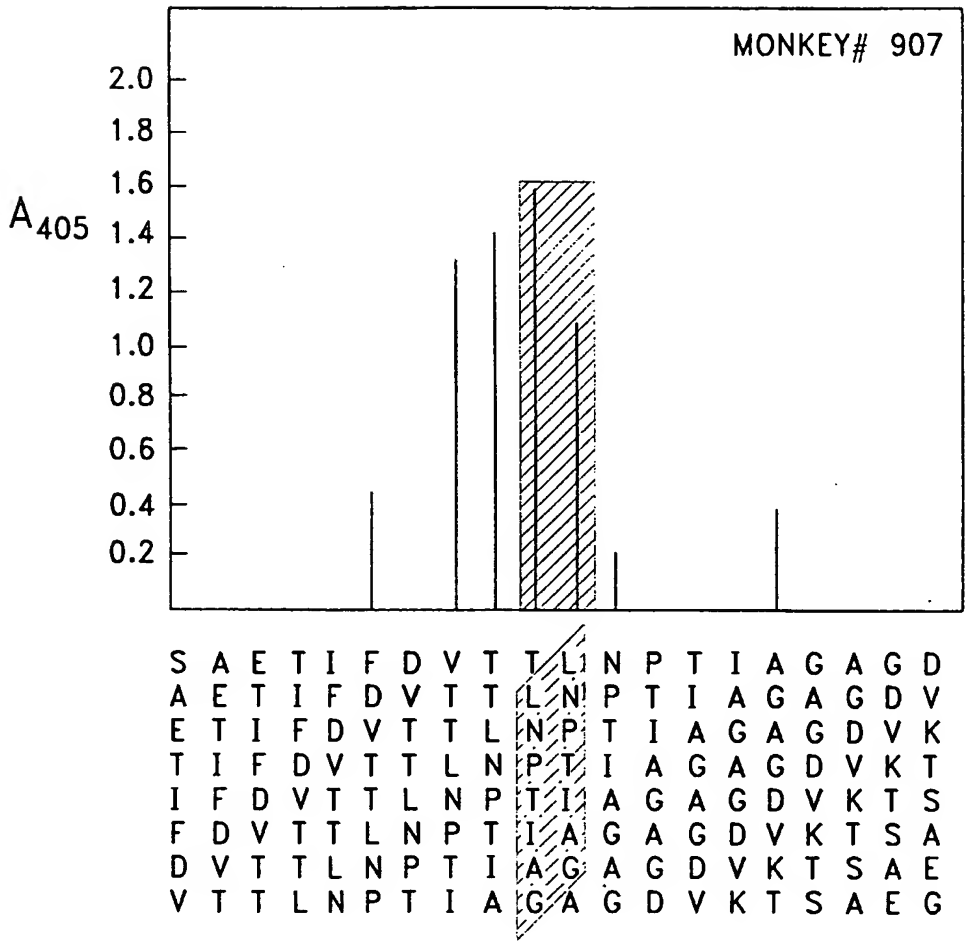
V T T L N P T I A G A G D V K T S A E G
VDIV (288-314)

FIG. 5C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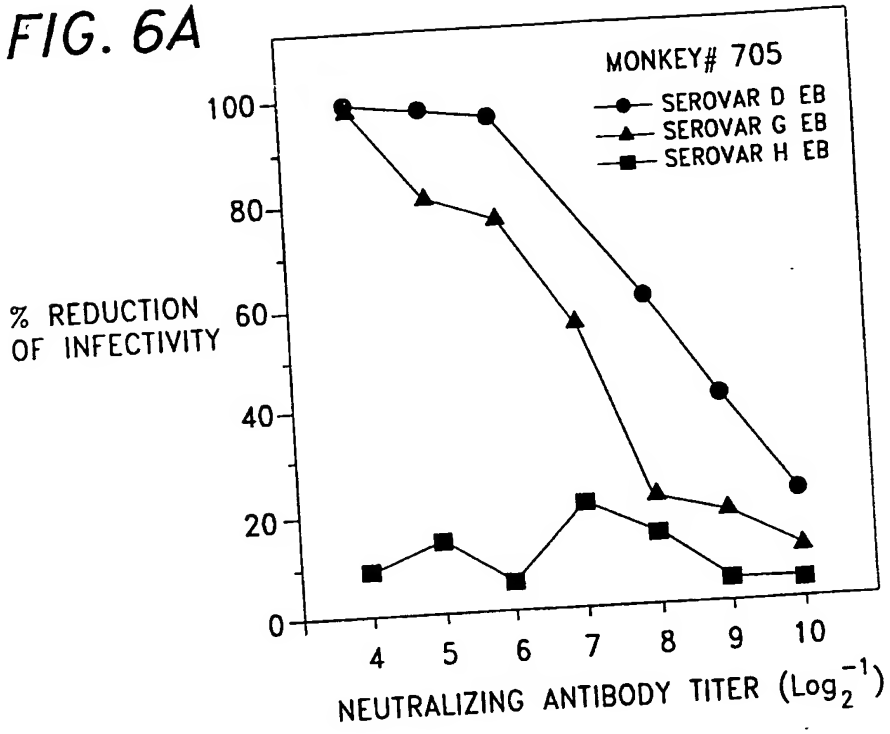
A E T I F D V T T L N P T I A G A G D V
E T I F D V T T L N P T I A G A G D V K
T I F D V T T L N P T I A G A G D V K T
I F D V T T L N P T I A G A G D V K T S
F D V T T L N P T I A G A G D V K T S A
D V T T L N P T I A G A G D V K T S A E
V T T L N P T I A G A G D V K T S A E G
VDIV (288-314)

FIG. 6A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5
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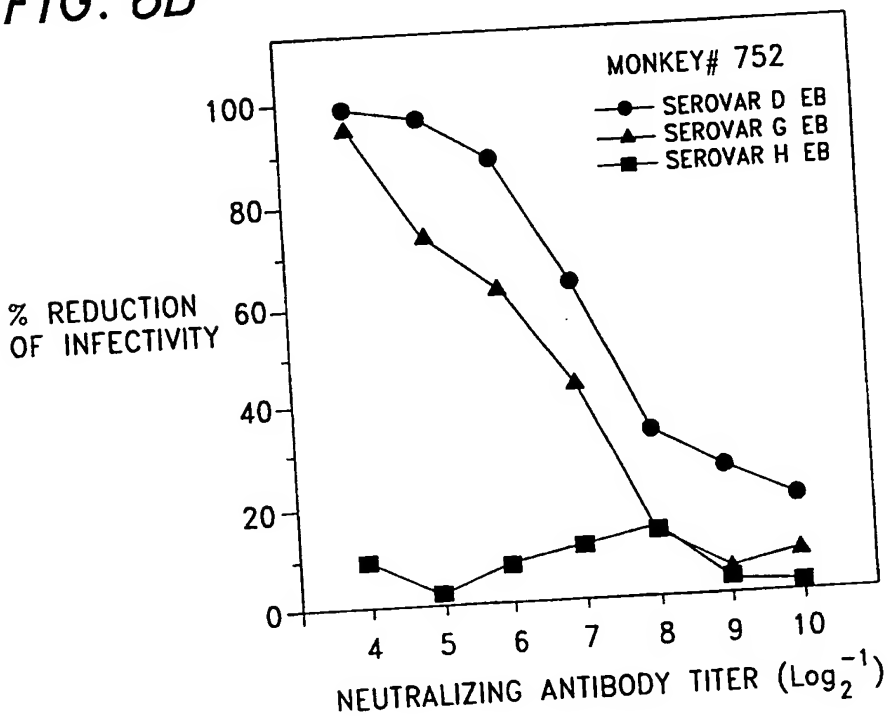
7
8
9
10
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 6B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% REDUCTION OF INFECTIVITY
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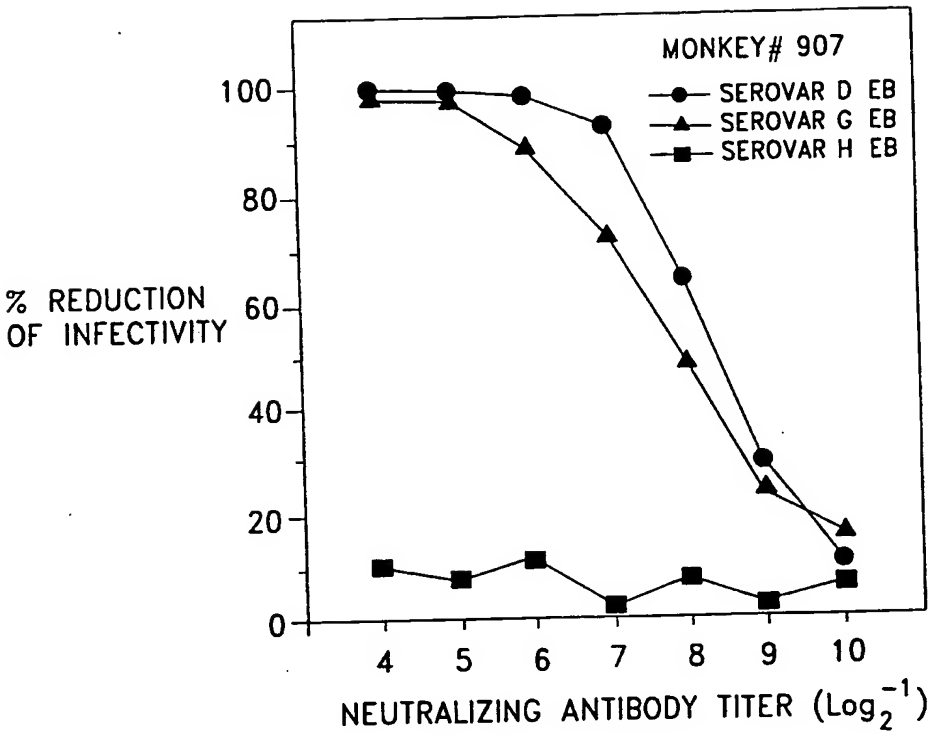
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 6C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SEROVAR H EB
% REDUCTION OF INFECTIVITY
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10
NEUTRALIZING ANTIBODY TITER (Log_2^{-1})

FIG. 7A1

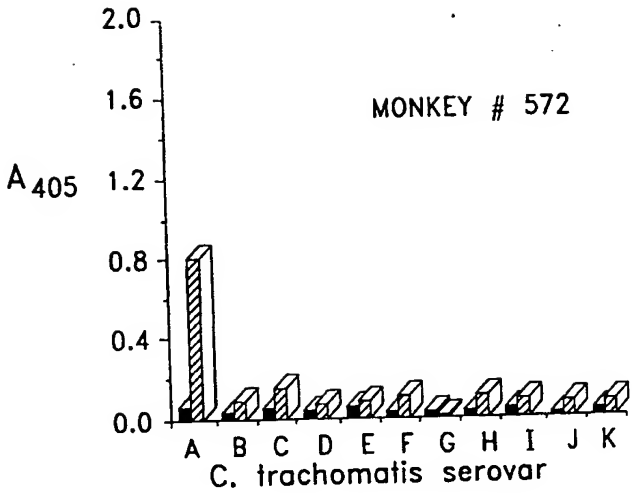


FIG. 7A2

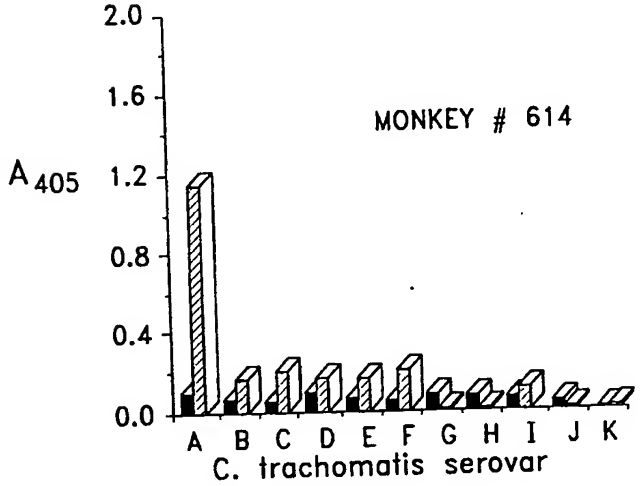


FIG. 7A3

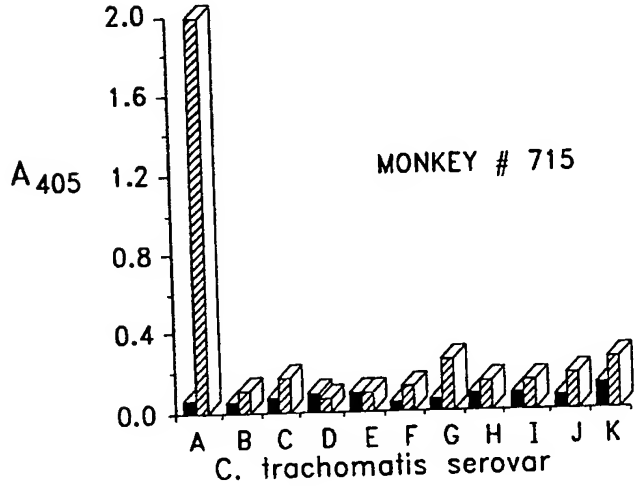


FIG. 7B1

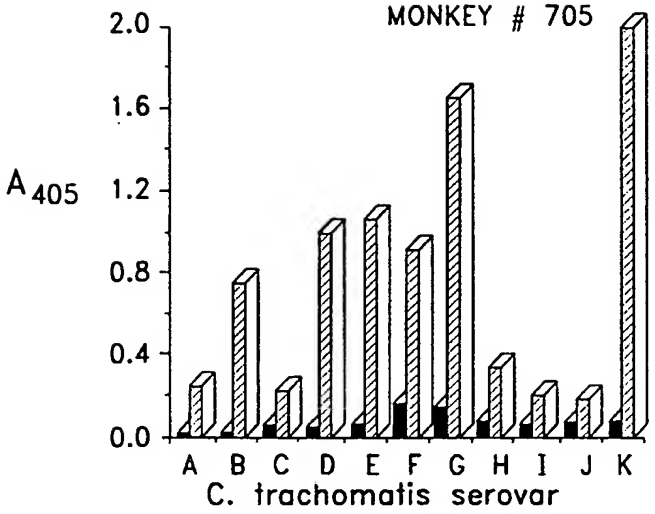


FIG. 7B2

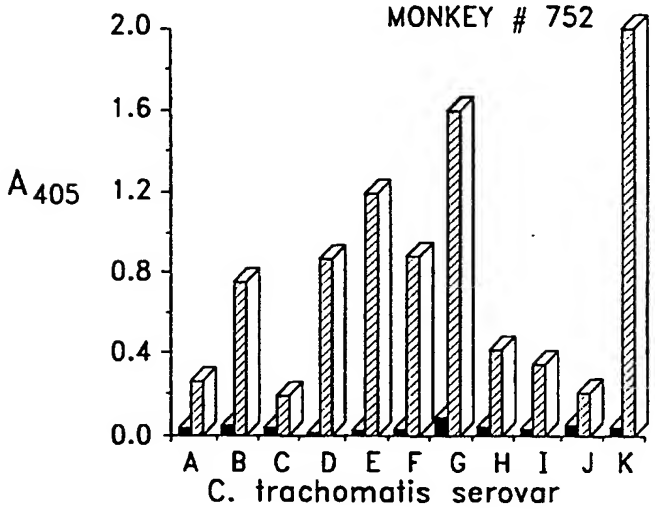
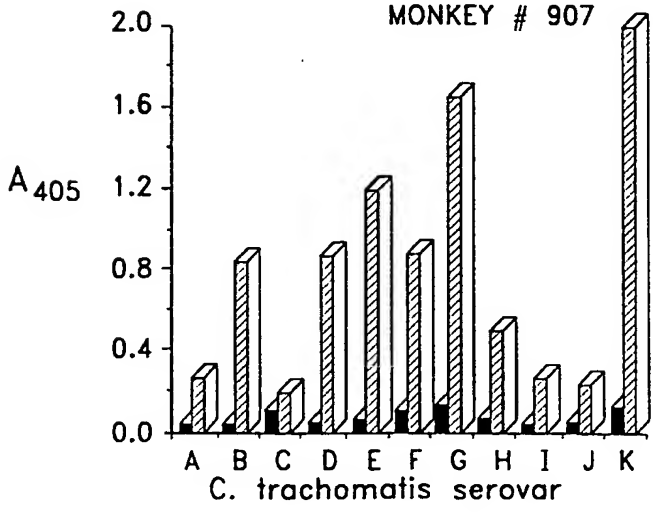


FIG. 7B3



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/08739

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^5: C 07 K 7/08,A 61 K 39/118,G 01 N 33/569

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC^5	C 07 K,A 61 K,G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
P,X	CHEMICAL ABSTRACTS, vol. 119, no. 8, issued 23 August 1993 (Columbus, Ohio, USA) H.D. CALDWELL "Synthetic peptide vaccine for Chlamydia trachomatis." pages 516-7, the abstract-no. 80199k & US Pat. Appl. US 947 671 01 April 1993 --	1-12, 14-16
A	EP, A2, 0 348 725 (MOLECULAR DIAGNOSTICS, INC.) 03 January 1990 (03.01.90), abstract; page 14; table 1. --	1-10
P,A	CHEMICAL ABSTRACT, vo. 119, no. 9, issued 30 August 1993 (Columbus, Ohio, USA) A.J. STAGG et al. "Primary human T-cell responses to the major outer membrane protein	11,12, 14-16

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
05 January 1994	21.02.94
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SCHNASS e.h.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	of Chlamydia trachomatis." page 873, the abstract-no. 93212j & Immunology 1993, 79(1),1-9 --	
P,A	CHEMICAL ABSTRACTS, vo. 118, no. 7, issued 15 February 1993 (Columbus, Ohio, USA) X. CHENG et al. "Characterization of the humoral response induced by a peptide corresponding to variable domain IV of the major outer membrane protein of Chlamydia trachomatis serovar E." page 634, the abstract-no. 57629w & Infect. Immun. 1992 60(8), 3428-32 --	11,12, 14-16
A	CHEMICAL ABSTRACTS, vol. 117, no. 5, issued 03 August 1992 (Columbus, Ohio, USA) H. SU et al. "Immunogenicity of a chimeric peptide corresponding to T helper and B cell epitopes of the Chlamydia trachomatis major outer membrane protein." page 697, the abstract-no. 46138p & J. Exp. Med. 1992, 175(1), 227-35 --	11,12, 14-16
A	CHEMICAL ABSTRACTS, vol. 113, no. 11, issued 10 September 1990 (Columbus, Ohio, USA) G. ZHONG et al. "Mapping antigenic sites on the major outer membrane protein of Chlamydia trachomatis with synthetic peptides." page 539, the abstract-no. 95768v & Infect. Immun. 1990, 58(5), 1450-5 ----	13

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

PCT/US 93/08739 SAE 79569

ANNEXE

au rapport de recherche international relatif á la demande de brevet international n°

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés á titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 348725	03-01-90	EP A3 348725 JP A2 3063296	24-10-90 19-03-91